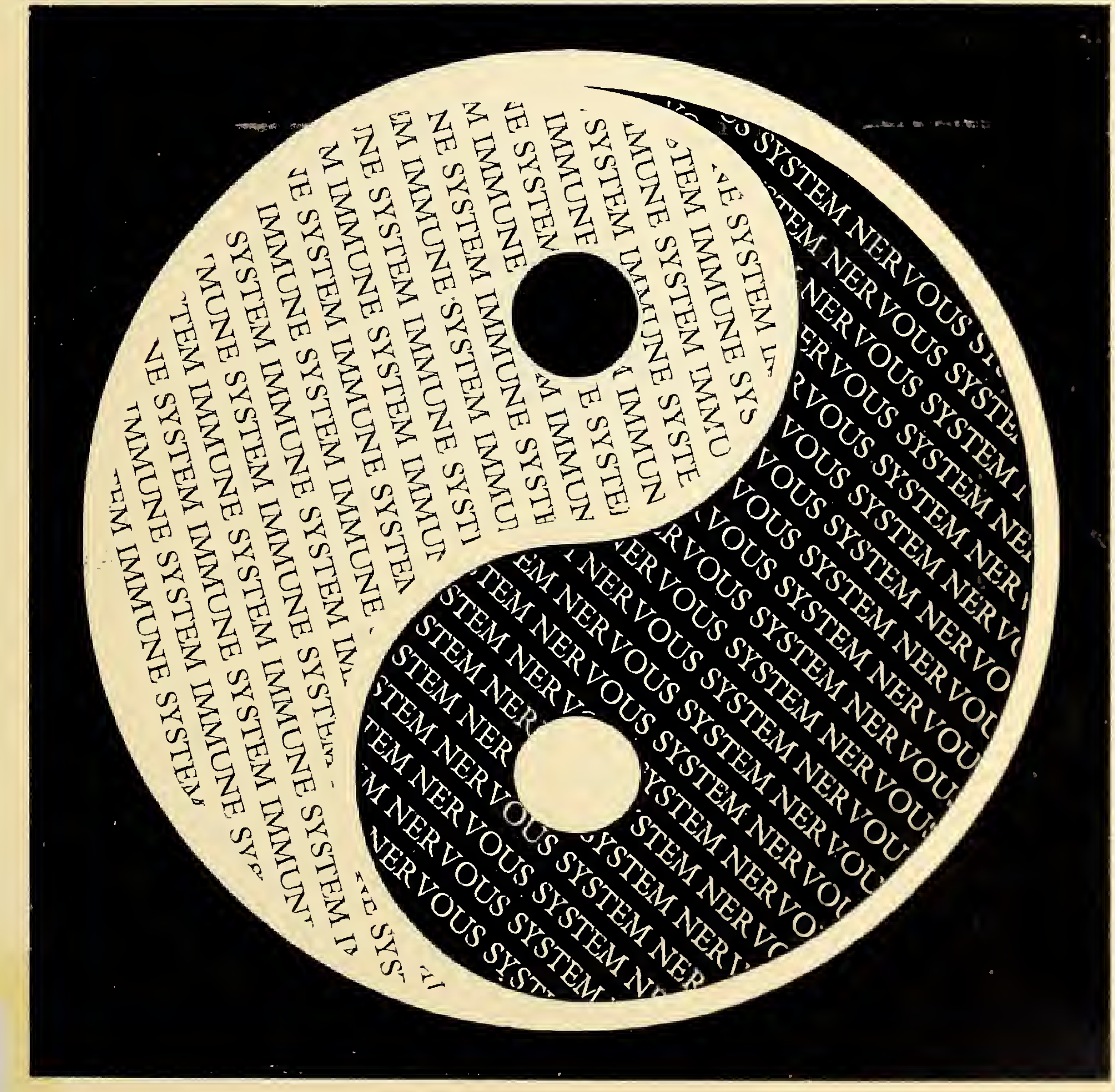
NeuroImmunology and Mental Health



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Neuroimmunology & Mental Health

A Report on Neuroimmunology Research

Edited by

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FOREWORD

We humans are restless creatures, always seeking new frontiers to conquer and greater challenges as we push the outer boundaries of our knowledge. One of the last great frontiers for exploration is our own brain. We have greatly increased our understanding of its fundamental operating systems in the last decade. Building on the fact that pharmacological interventions effectively treat mental illnesses, we are exploring and gaining an increased understanding of the neurobiological basis of these disorders and working to understand their genetic and molecular bases.

Many of these increments in knowledge have resulted from technological advances in other fields, such as computer technology, medical imaging, and molecular biology. This newly discovered information—especially in the emergent field of neuroimmunology—has contributed significantly to the decline of the postulated mind/brain dichotomy and the rise of a new and richer conception of the physical bases of emotions and cognition.

Of all the scientific fields of study within neuroscience, neuroimmunology is perhaps the youngest. In fact, only recently was it definitely shown that the brain is immunologically active. The field of immunology itself has rapidly become quite sophisticated, with significant definition of the molecules and mechanisms of the immune system.

The time is ripe to study more closely the relationships between the immune system and the brain. Most of our organ systems are regulated by the brain. Historically, it has been believed that the immune system is independently and flexibly poised to protect the body, reacting only to foreign invaders. This dogma is currently questioned by data showing immune system regulation by the brain and suggesting that the brain is influenced by the immune system, resulting in interactive systems. While the controversy continues over this question, the evidence seems to support brain/immune system interactions, but it also implicates a third component—the endocrine system.

To the National Institute of Mental Health, a fourth critical component of this puzzle is the role of behavior, specifically emotions and cognition. The brain is an extremely sensitive detector of both the internal and external milieus. When we suffer from a bacterial or viral infection, we may feel fatigued, sleepy, anergic, or depressed, and our cognitive ability may falter. Are these symptoms simply due to fever? Viral infections rarely result in fever, raising the question of the etiology of these behavioral effects. One could postulate the etiological agent to be viral products, cytokine production in the brain, or the activation of macrophages in the brain. Could a virus actually precipitate the onset of a depressive episode or cognitive impairment?

We have recently discovered that some individuals infected with the human immunodeficiency virus (HIV) show impaired cognitive function during early asymptomatic stages of acquired immunodeficiency syndrome (AIDS), and many more cases of full dementia are seen during the latter stages of AIDS. Viral infections have been implicated as well in the etiology of schizophrenia. Many epidemiological studies have shown a relatively high rate of schizophrenia in adults who, during the second trimester of their prenatal development, were exposed to the influenza virus.

Complementing these studies are data supporting the theory that immune system function can be improved by enhancing coping skills. An initial well-controlled trial found increased survival time among breast cancer patients who participated in group psychotherapy compared with nonparticipants. While quite controversial when first reported, this finding has been independently reproduced by a second group of investigators and is being retested by the original research team. Growing evidence thus supports a critical place for behavioral factors in the complex set of interactions among the immune, endocrine, and the central nervous systems.

As documented in this report, our knowledge of the neuroimmune and neuroendocrine mechanisms mediating the relationship between mental state, behavior, and health and disease has reached a critical state. The progress to date now begs for increasing our efforts to understand these powerful systems and to unravel their mechanisms of interaction. Crucial to these new efforts is the continued development of research tools. But the most rate-limiting factor at present is the need to develop better experimental approaches and theories to unify the existing data. We now know that molecules and interactions involved in the bidirectional communication between the brain and immune system are redundant, overlapping, and exquisitely fine-tuned.

The goal of this report is to educate and stimulate thinking about the mechanisms enabling these four systems to interact. Ultimately, it should promote research that will further advance understanding of how the brain influences and is influenced by the systems germane to maintaining homeostasis and to defending and repairing the body's tissues. That research will clarify the interrelationships between behavioral states and physical health. Finally, when applied, our new knowledge about neuro-endocrine-immune-behavior interactions should improve both physical and mental health.

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PREFACE

Exciting breakthroughs in neuroscience and immunology are confirming the influence of mind over body and the effect of physical health on mental state. Scientists are now proving the powerful impact of attitudes, emotions, and friendships on the course of disease. Distinctions between the nervous and the immune systems are breaking down, as researchers discover similar types of cells and molecules performing similar functions in both systems, and discern a complex communication system connecting them.

The relatively new science of psychoneuroimmunology is looking at the human organism—mind and body—as a whole and is making fascinating discoveries that promise better approaches to ensuring both mental and physical health. This multidisciplinary approach has provided new insights into the causes of multiple sclerosis, schizophrenia, chronic fatigue syndrome, human immunodeficiency virus infection, and a variety of viral insults. Researchers are already testing a novel treatment for Alzheimer's disease.

Psychoneuroimmunology research also examines how group and individual psychotherapies can be used to treat physical illness, such as breast cancer. Changing behavior can diminish, relieve, or modulate various environmental and perceived stresses that weaken our immune systems. On the other hand, some stresses strengthen our immune system and could be used to our advantage.

This report is designed to inform the lay and scientific communities about this extraordinary progress in the field of psychoneuro–immunology and to promote research that will further advance our understanding of how the brain influences, and is influenced by, the various systems that maintain homeostasis and defend/repair the body's tissues. Thus, the report contains specific recommendations for applying knowledge about neuro-endocrine-immune interactions to the improvement of behavioral and medical outcomes.

Developing and supporting neuroimmunology research is the specific mandate of the Neuroimmunology/Neurovirology Program within the Division of Neuroscience and Behavioral Science (DNBS) of the National Institute of Mental Health. The formation of the Program provided a unique opportunity for defining and conceptualizing those areas of neuroimmunology research that have the greatest potential for contributing to mental health.

In order to accomplish this goal, DNBS sought the expertise and guidance of distinguished scientists in several disciplines. These scientists participated in a series of workshops held between July 1992 and July 1993. They sought to determine the current level of knowledge in the field, identify research directions most likely to yield important discoveries during the next 5 years, identify biological processes potentially amenable to therapeutic interventions, and formulate questions that should be answered in the next 10 years.

This report is the product of their collective activity. The contributions of members of the scientific panels, named on the inside cover of this volume, are gratefully acknowledged. Appreciation and gratitude are also expressed to Joe Alper and Jane K. Myers, who unrelentingly pursued the overwhelming task of combining the individual and group reports from the scientific panels.

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THE BRAIN AND IMMUNITY

"We're going to see a lot of commonalities between these two systems."

—Michael B. Prystowsky

The notion that the mind can affect overall health is not new. Ancient Eastern writings, particularly from India, espouse a healthy mind to keep the body robust. Nearly two millennia ago, Galen, a Greek physician, wrote that melancholy women are more susceptible to breast cancer than are cheerful women. A number of modern investigators have reported that positive mental attitude improves survival rates in cancer patients.

The scientific literature is filled with both anecdotal and concrete evidence that the mind has a powerful influence on the body. Stresses of all kinds—mental and physical—can reduce the body's ability to fight disease, yet a positive outlook can help patients battle even the most serious illnesses, including acquired immune deficiency syndrome (AIDS) and cancer.

The mind has a powerful influence on the body.

This connection between the mental and the physical works both ways. Infection in the body has well-known effects on behavior and mental state. Everyone is familiar with the lethargy, gloom, and irritability that accompany a cold or flu. Patients infected with the human immunodeficiency virus (HIV) often show subtle behavioral changes before any other symptoms appear. As they develop full-blown AIDS, subtle behavioral problems often turn into debilitating dementia.

Today, spurred on in part by the tragedy of AIDS and also by the progress made on a number of scientific fronts, researchers from a wide variety of disciplines are giving new meaning to the old adage, "Laughter is the best medicine."

A Multidisciplinary Field of Study

Neuroscientists and immunologists, along with molecular biologists, virologists, epidemiologists, developmental biologists, and others, are uncovering the myriad connections between our mental and physical states. Armed with a host of new biochemical, genetic, immunological, and imaging tools developed over the last two decades, these researchers are pushing the boundaries of psychoneuroimmunology—the study of the interrelationships among behavior, the nervous system, and the immune system.

Heretofore, researchers have focused on individual systems in the body, seeing them as distinct, self-contained, and self-regulated entities. Now, they are finding that the nervous and immune systems have much more in common than previously thought, work together efficiently, and are in constant communication with each other (fig. 1). Psychoneuroimmunology focuses on this integrated, interacting whole.

Studies of the parts do not necessarily reveal the whole.

Investigators in this relatively new field are struggling with the ever-present problem of having to conduct most of their experiments on bits and pieces—cells and molecules—or on specially bred animals. *In vitro* studies involve isolating a type of cell, gene, molecule, or other entity, placing it in an artificial environment, and watching how it reacts under a variety of exposures and conditions. These findings then have to be related to the much more complex human body. For example, new chemical mediators are being discovered in the laboratory that appear to play important roles in activating both the immune system and the nervous system. Now investigators must determine if these substance perform the same way *in vivo*—in the body—where they may be parts of several complicated systems and interact with numerous other chemicals. Psychoneuroimmunologists happily embrace such challenges, for this type of intellectual obstacle drives science forward.

Three Interacting Components

To appreciate the interrelationships among behavior, the nervous system, and the immune system requires some understanding of each entity alone. Their differences in structure and apparent function make it easy to see why they were studied independently for so long.

Behavior

Thought and behavior still mystify us. The connection between intelligence, emotion, and the body is so elusive that separate concepts are used—an ephemeral thing called the mind and a concrete, physical thing called the brain. Whether the mind controls the brain or vice versa is beyond the scope of this report.

Behavior arises from both internal and external cues. For example, individuals may be so engrossed in thinking about something that

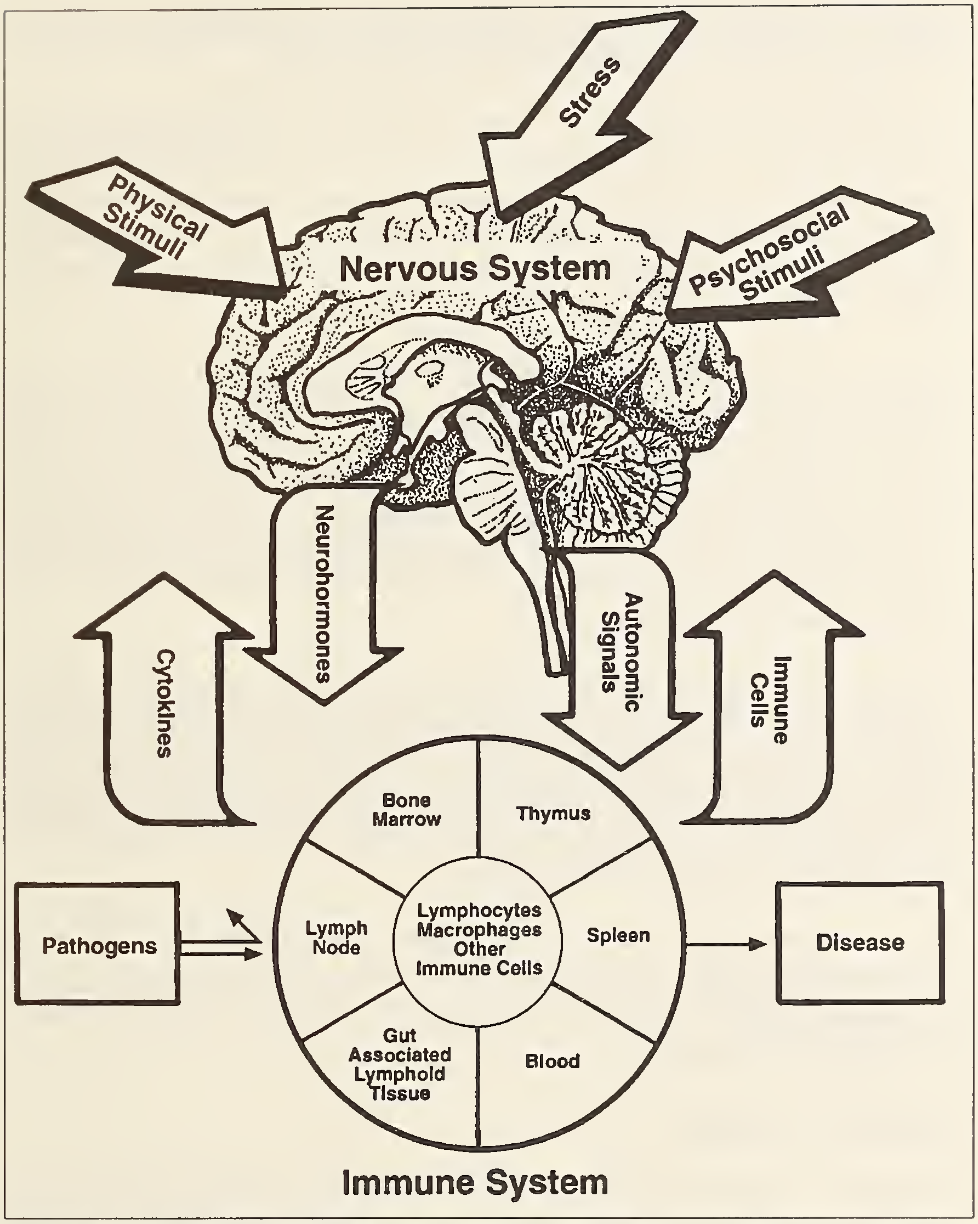

FIGURE 1.—Bidirectional communication between the nervous and immune systems. Some organs comprising the two systems interact to ward off pathogens and prevent disease while the organism is responding to external stimuli. The unimaginable complexity of the interactions is being slowly but surely unravelled at the cellular and molecular levels. [After D. Felten.]

nothing disturbs their train of thought, or so distracted by whatever is going on around them that they cannot think clearly. Emotions may be so strong as to seem out of control.

Extreme disorders in thought and mood are labeled mental illness, and some success in treating them has been attained through the body by giving the patient various chemical substances. Some depressions have been controlled by light therapy.

Supportive people help alleviate distress.

Some mental and emotional distress is alleviated by the presence of caring people. Loving caregivers and support groups made up of people sharing the same problem have brought noticable improvement in many mental and physical conditions. Sometimes, simply the presence or absence of family or friends is the determining factor.

Things that impinge upon a person's present mental or emotional state are lumped together under the generic term "stress." These can be positive or negative events and can arise from within the person or come from the environment. The effects of stress depend on the nature of the stress and its duration. Being startled by something that turns out to be nonthreatening will have little or no impact, while facing an ongoing, serious threat for an appreciable time can be quite deleterious.

Positive stress can have beneficial effects.

On the other hand, positive stress, such as preparing for a happy event like a wedding, can have positive effects on mental and emotional well-being. Athletes under the stress of competing in an important, major event can become so incredibly focused that play seems automatic, and they perform better and more effectively than ever before (or since) in their lives.

Several approaches have been used to alleviate the effects of longstanding negative stress. These include prescribing drugs such as tranquilizers, teaching relaxation techniques, and providing group psychotherapy.

The Nervous System

The brain, spinal cord, and peripheral nerves—those branching out from the spinal cord to various tissues and organs—are the chief components of the nervous system. Part of the peripheral nervous system, called the autonomic system, controls the internal organs. The peripheral system relays commands from the central nervous system—the brain and spinal cord—to the rest of the body and conveys information about the body and the external world to the brain. While cells in the central nervous system also perform such communications functions, they have integrative and analytic abilities that cells in the peripheral nervous system lack.

Neurons are vastly overproduced during development.

The two main classes of cells in the nervous system are neurons and glia. Neurons send and process information in the form of electrical and chemical signals. Glia have a host of support functions. During development, far more neurons are produced than are needed in the mature brain, and about 40 percent of them die. Many theories have been put forth for this normal neuronal death, but neuroscientists still do not fully understand the reasons behind this overproduction.

Neurons

A neuron consists of a cell body, containing the nucleus and metabolic apparatus, and long extensions, much like the branches of a tree, called dendrites. Also projecting out of the cell body is a single fiber called an axon, which can be 3 or more feet long in humans and has many terminals branching from its end. Information, in the form of an electrical pulse, comes into the nerve cell via the dendrites and goes out from the cell through the axon, which passes that signal to the dendrites of neighboring neurons. Large axons are surrounded by a fatty sheath of myelin, which acts as an insulator.

Neighboring neurons do not actually touch but are separated by a thin gap known as the synaptic cleft. Electrical signals cannot cross this gap, so the neuron releases a chemical, called a neurotransmitter, from a terminal at the end of the axon. Neurotransmitters diffuse across the synapse and attach themselves, or bind, to sites (receptors) on the neighboring dendrites that will accept only that specific type of chemical. This binding triggers a chain reaction within the dendrite that produces an electrical signal that moves up the dendrite to the body of the neuron.

Electrical signals are transmitted from neuron to neuron by chemicals.

Other compounds, known as neuropeptides and neuromodulators, are also released from terminals of the axon. These substances modulate or attenuate the effects of the primary neurotransmitter active at a particular synapse. Once the signal has been passed, the neurotransmitter is taken up by the dendritic nerve terminal, while neuropeptides and neuromodulators are cleared from the synaptic cleft by neighboring glia.

Glia

The central nervous system has between 10 and 50 times more glial cells than neurons. Glia are smaller than neurons and do not generate electrical signals. Like neurons, however, they secrete a host of chemicals that trigger actions by other cells. Some of these chemicals may prove to be critical links between neurons and the immune system. Most glia provide structural support for neurons—a scaffolding upon which neurons grow and make connections with one another.

Glial cells vastly outnumber neurons.

Glial cells come in several forms, and the functions of many of them are yet to be discovered. Astrocytes, oligodendrocytes, and ependymal cells are large cells known collectively as macroglia. One known function of oligodendrocytes is to form the myelin sheath insulating axons. Astrocytes absorb toxic substances in the fluid surrounding neurons and produce biochemicals, such as neurotransmitters, that enable the neuron to function (fig. 2). They are the most important regulators of homeostasis in the brain. Astrocytes are capable of displaying various phenotypes when examined in isolation, but their precise phenotypes in the living body are unknown.

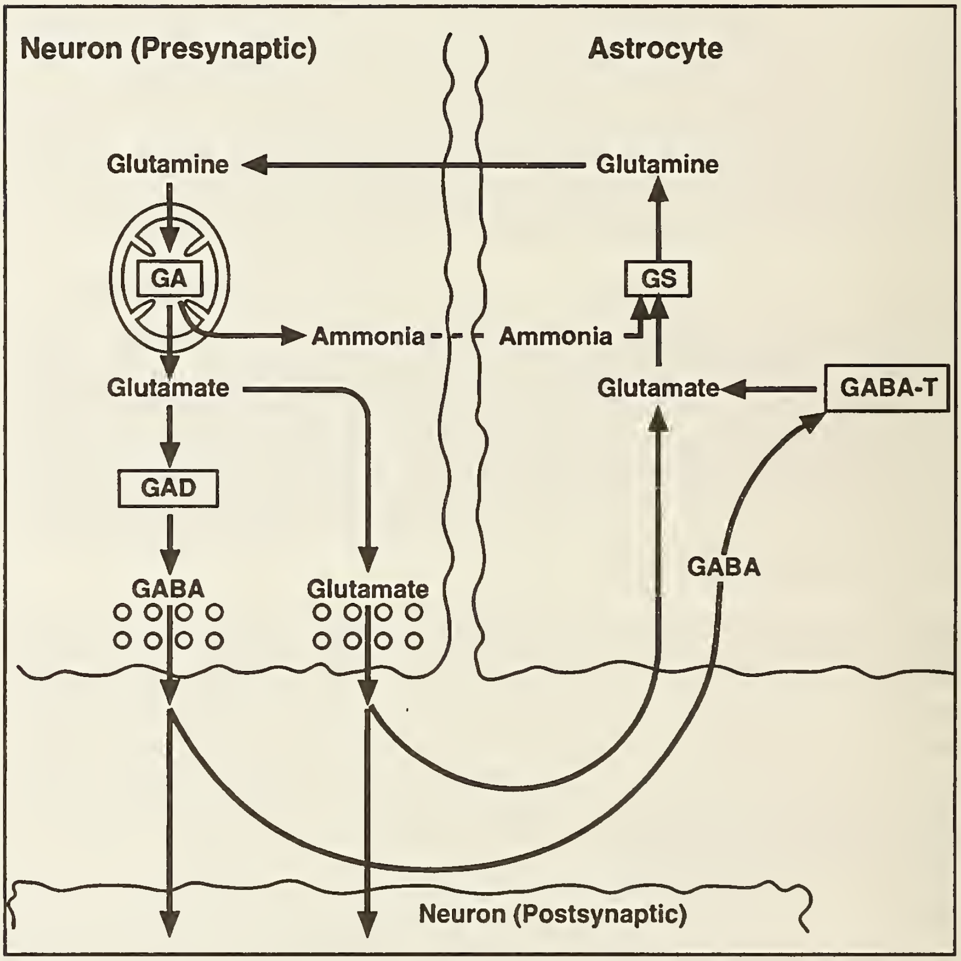

FIGURE 2.—How astrocytes facilitate neuronal functioning. Astrocytes synthesize glutamine using ammonia, a toxic substance, in the brain. Ammonia is removed from the body by biochemical reactions called the urea cycle, which does not operate in brain. Glutamine is taken up by neurons and converted into glutamate, a major excitatory neurotransmitter. Glutamate is then used either for neurotransmission or converted into gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter. Excess neurotransmitter molecules, if present in the synaptic cleft, are "mopped up" by astrocytes. Enzymes catalyzing these reactions are compartmentalized between astrocytes and neurons. They are glutamine synthetase (GS), glutaminase (GA), glutamate decarboxylase (GAD), and GABA transaminase (GABA-T).

Microglia include a variety of cells capable of engulfing and digesting cellular debris after injury, infection, or disease. These cells are also capable of moving through the brain under certain circumstances.

The Immune System

The immune system is made up of cells and molecules whose main function is to distinguish the body (self) from foreign matter (non-self) and to defend against foreign organisms or substances. The lymphoid system, including the spleen, thymus, and bone marrow, plays an important role in the development of immune system cells (lymphocytes), and these cells produce many of the molecules that detect and respond to invaders. Lymphocytes may further develop into helper cells, suppressor cells, or killer cells.

The immune system can attack the body's own tissues.

On occasion, the mechanisms that control the ability to discriminate between self and non-self fail; immune tolerance (nonreaction) is lost, and the immune system attacks the body's own tissues. This failure is known as autoimmune disease. In addition, damage to healthy tissue can occur in the normal process of eliminating pathogens.

When the immune system detects non-self, it can respond in a variety of ways. The complement reaction, for example, consists of a complex series of biochemical steps (a cascade) involving a number of molecules circulating in the blood—and recently detected in brain tissue—that among other things poke holes in an offending cell and kill it. Large phagocytes (cells that engulf and digest) called macrophages migrate to the site of an infection or injury and capture the material that requires elimination. Complement proteins and macrophages, along with other cells called neutrophils and monocytes (the precursors of macrophages), are components of the nonspecific immune response (fig. 3).

The humoral immune response is characterized by the production of molecules called antibodies. Antibodies are produced by cells called B-lymphocytes that are formed and matured in the bone marrow. These cells usually react when they encounter particular molecules, called antigens, on the surface of invading bacteria and viruses. Each B-lymphocyte and its offspring recognizes and responds to one type of antigen by producing antibodies that bind to, or neutralize, that antigen. The antigen-antibody reaction triggers other immune system cell activity that culminates in the destruction of the cell or organism containing the antigen.

Cell-mediated immunity is carried out by immune cells called T-lymphocytes that start out as progenitor cells in the bone marrow and

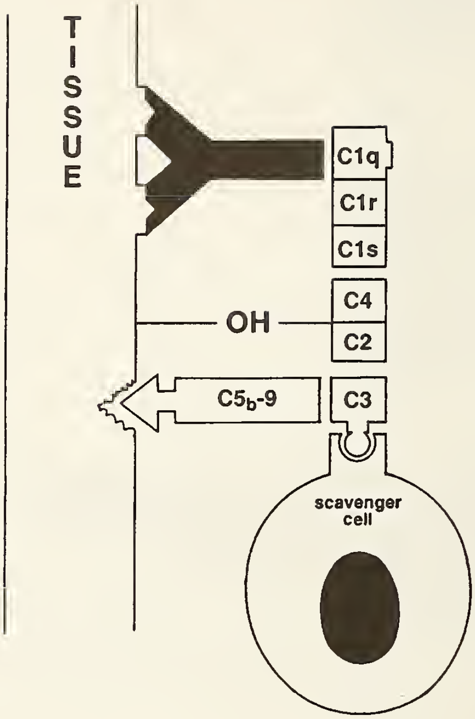

FIGURE 3.—Schematic diagram of various interactions between diseased tissue components and complement proteins. The first interaction is mediated by an antibody molecule (in black) binding to an antigen in the tissue, and complement proteins C1q, C1r, and C1s (top). The second interaction is between -OH groups on proteins in the tissue and the complement proteins C2 and C4 (middle). The third interaction is between the membrane of a cell comprising the tissue and complement proteins C5b–9, called "membrane attack complex" (bottom). Activation fragments of C3 serve as chemotactic signals for a scavenger cell, such as a macrophage, microglial cell, or possibly an astrocyte, to approach diseased tissue and remove the damaged parts. Complement proteins may mistakenly facilitate healthy brain tissue destruction (arrow).
[Courtesy J. Rogers.]

mature in the thymus. T-lymphocytes act against viral and fungal infections, reject grafts of foreign tissue, and fight malignancies. T-lymphocytes themselves, along with all the other cells of the immune system, produce a variety of biologically active substances known as cytokines, which include interferons and interleukins.

Key players in both humoral and cell-mediated immunity are the members of the major histocompatibility complex (MHC) of gene products. These large molecules—proteins with attached sugars—are found on the surfaces of many types of cells and play a role in signaling B-lymphocytes and T-lymphocytes. There are two classes of MHC molecules. MHC Class I molecules are found on virtually all cells in the body, though there is still some question as to how widespread they are in the central nervous system. These molecules help T-lymphocytes recognize cells infected by a virus. MHC class-II molecules are confined largely to immune system cells and play a critical role in the recognition of foreign antigens by both B-lymphocytes and T-lymphocytes.

Two Systems With Many Similarities

It is a common observation that nature is redundant: what works in one situation, whether it be a behavior or a chemical reaction, is often used with only slight modification in another situation. Nowhere is this more true than in the nervous and the immune systems. Certainly, the brain is unlike the thymus or bone marrow, and neurons and glia differ in many significant ways from lymphocytes and macrophages. Nevertheless, both systems engage in a constant survey of the local environment—internal and external—and both analyze and respond to constantly changing conditions.

Moreover, research is continually turning up ways in which these two systems are more similar than different, at both the cellular and molecular levels. For example, these are the only two systems endowed with the ability to "remember" and "learn" from their experience and then modify their "behavior" based on past exposure.

Cells With Similar Functions

In the nervous system, neurons may be the main actors, but they do very little without the support of glia. Similarly, T- and B-lymphocytes are in many respects the cornerstone cells in the immune system, yet they could not function without the support of macrophages.

The similarities go beyond this simple analogy. Until recently, microglia were largely a mystery, but now there is increasing evidence that these normally quiescent cells likely play the same role in the brain that macrophages play outside the central nervous system: scavenging cellular debris. When neurons are damaged, during infection or following physical trauma such as stroke, microglia appear to interpose themselves between synaptic processes and the neuron cell body. There, they clear the site of cellular fragments by the same process that macrophages use, while also releasing cytokines such as interleukin-1, which may stimulate a variety of actions within both the brain and the immune system.

Shared Molecules

Interleukin-1 is just one example of a molecule used by both the nervous system and the immune system. In fact, many scientists now argue that neurotransmitters, the molecules used by nerve cells to communicate with one another, and cytokines, the molecules used by immune cells to communicate with one another, are all part of a complex, but unified, system of chemical communication.

The body may have a unified system of chemical communication.

Cytokines and neuropeptides, for example, both bind to specific receptors thought to be on only one kind of cellular surface. But research has also found neuropeptide receptors on the surfaces of lymphocytes and other immune cells, while neurons and glia have cytokine receptors.

This is not to say that these molecules and receptors have identical functions, but it does suggest that they may share common chemical pathways, extending perhaps to the level of gene expression, that ultimately produce a tissue- or cell-specific response appropriate for a particular environment. Examining these possibilities could lead to a unified theory of biological signaling, which could have profound implications for treating infection, inflammation, tumor development, mental illness, and other immunological and neurobehavioral problems.

MHC molecules, key players in the immune system, are found in the nervous system on microglial and perivascular cells. In some areas of fully developed pathology, a few astrocytes may express MHC molecules.

The same genes have been found activated in both T-lymphocytes and neurons.

Only recently, researchers have discovered one of the latest in a group of genes that are activated in both T-lymphocytes and neurons exposed to cytokines. This gene, named F5, responds to interleukin-2 stimulation by producing a protein of still unknown function in growing, or proliferating, T-lymphocytes as well as in all areas of the brain, particularly where neurons have long axons and many connections, such as the hippocampus, motor cortex, and cerebellum. Furthermore, F5 is active only in neurons, not glia, and its expression is greatest when neurons are forming synapses with other neurons rather than when the neurons are replicating.

Plasticity

One of the hallmarks of the immune system is its ability to adapt to constantly changing conditions and to remember how it responds to each of those conditions. Much of this memory is expressed in the way the immune system responds to antigens that it has seen before.

The nervous system is constantly changing.

Conversely, the nervous system was long thought to be static, with little ability to change once its billions of neurons were in place. One of the more important discoveries in neuroscience was that the brain, too, is constantly changing and adapting in response to changing conditions. For example, it is now clear that behavioral modification during learning results from functional changes in the connections between nerve cells and in the way they respond to neurotransmitters.

Behavior and Immunity

The connections between behavior and the immune system are perhaps best established in the body's response to stress. Research has clearly shown that psychological stress can lead to increased susceptibility to disease by suppressing the immune response. Yet, studies in both humans and laboratory animals show that mild stress actually increases the activity of various immune cells. In one set of experiments, researchers subjected animals to a mild stress and found that natural killer cell activity, interleukin-2 production, and other measures of immune system activity increased over the next hour.

Even when the stress is moderate to severe, changes in immune cell function do not always translate into increased susceptibility to illness. However, researchers studying the effect of chronic stress, such as long-term caring for a relative with Alzheimer's disease, found decreased immune cell function and increased incidence of illness in the caregivers; when the stress was removed, immune system function did not always return to normal. Clearly, much more research is needed to explain this range of responses.

Stress and Susceptibility to Disease

Considerable evidence from studies of humans shows that naturally occurring stressful events and negative moods such as anxiety and depression are associated with changes in immune function. Whether these changes have implications for susceptibility to infection and other immune-mediated diseases is less clear.

A recent study examined whether persons experiencing stress were at greater risk for developing a common cold. Volunteers were exposed to nasal drops containing one of five viruses known to cause a cold. Before exposure, the subjects filled out three questionnaires designed to assess their current level of stress. A life-events scale measured the occurrence of stressful events—divorce, death of a relative, problems at work or school, and the like. Another scale gauged perceived stress—feeling that the demands of life exceed one's ability to cope. A third measure assessed negative moods, including depression, anxiety, and anger.

People experiencing stress are more apt to catch a cold.

The researchers found that the higher the level of reported stress, the greater the probability of developing a cold. This association held whether the three stress measures were considered alone or together and across all five viruses tested (fig. 4). Although functional measures of immunity were not assessed in this study, researchers are currently examining the possible role of immune factors in linking stress to infectious susceptibility.

Medical students were studied to compare immune system function during intermittent periods of stress (comprehensive examinations) and no stress. These studies gathered data on both psychological factors and immune measures, and the results show clearly that these students, though they are expert test-takers, have significant downregulation (slowing) of the cellular immune response at examination time and recover immune function after exams are completed. The one caveat of these studies is that all indicators of immune function were measured in vitro, which may not correlate with the actual overall health of an individual. However, a correlation was found between the time of examination, negative changes in the immune parameters, and upper respiratory tract infections.

Intermittent stress has intermittent effects on immunity.

Since some people handle stress better than others, investigators refined their analysis and looked at how different levels of anxiety, one measure of the psychological response to stress, correlated with

Ability to cope with stres affects immune respons

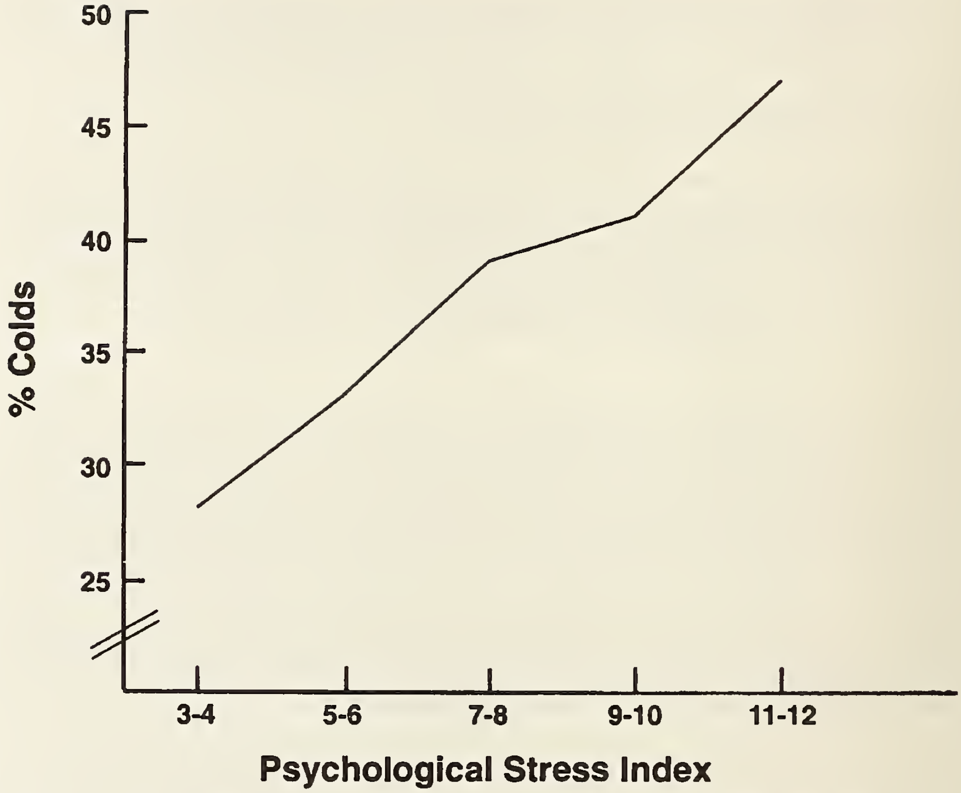

Figure 4. Susceptibility to a cold is directly proportional to the intensity of psychological stress. Healthy volunteers were exposed to one of five viruses known to cause colds. The probability of getting sick increased with the severity of psychological stress the person reported experiencing. [Courtesy S. Cohen.]

immune system response to a specific challenge, such as exposure to hepatitis B vaccine. In this case, the more anxious medical students had a smaller immune response to vaccination than did students who were less anxious.

This finding suggests that vaccinations may be less effective if administered when a person is feeling the effects of stress. For example, soldiers about to go oversees—a potentially stressful situation—are often vaccinated, but they may not build up full immunity as rapidly as under more relaxed circumstances.

The Protective Effects of Social Support

One clinically relevant finding of the medical student vaccination study was that the low anxiety group, who had the strongest immune response to vaccination, also had the highest level of social support from their peers. This suggests that providing social support to those vulnerable to stress may be an effective means of improving overall health.

Affiliation with others improves overall health.

More direct evidence for the beneficial role of social support comes from a study of the effects of social environment on the cellular immune response of monkeys. After 2 years, animals whose social groups were continuously disrupted by the researchers had poorer T-cell response to experimental stimulants than animals whose social environments were stable over the course of the study. However, when the investigators distinguished between individual animals who were affiliated with other animals and their more isolated counterparts, the picture changed. The immune suppression found among the socially disrupted group occurred only among the more isolated animals. Affiliative animals showed no immunosuppressive effects of the unstable social environment.

Unanswered Questions

A new model of stress is needed.

Researchers are now trying to determine an appropriate model for judging the effect of stress—or any environmental change—on the variety of immune responses. Some theoretical structure is needed to make sense of the wealth of data now being generated in the Nation's laboratories. These findings raise important methodological and clinical questions: What is an appropriate model for judging the effect of environment and stress on a variety of immune responses? Is mild, intermittent stress more relevant than chronic, severe stress? Is *in vitro* immune cell function a relevant measure of the immune system's ability to fight disease? Does the immune system function best under some optimal level of stress?

We have no clear answers to these questions, but investigators are beginning to come to grips with these issues, and their awareness will certainly lead to future advances in this important area of research. They may have to develop a new model for environmental pressure on an organism that will be more useful scientifically than the nebulous concept of stress.

Maintaining Homeostasis

The many ways the nervous and immune systems communicate with one another give the body its remarkable ability to maintain itself in equilibrium while existing in an ever-changing environment. This robust homeostasis may actually define life, as every minute of every day the human body attempts to maintain this precarious state despite the ever-present burden of inherited and acquired biochemical defects, including those induced by infectious organisms.

Homeostasis may define life.

Thus, a major goal of psychoneuroimmunology is to understand the psychobiology of defense and repair. Learning how the mind, via the nervous system, affects all of the systems that defend or repair the body—and is in turn affected by them—may provide significant breakthroughs in treating psychiatric, neurological, and immune disorders.

Recommendations for the Future

- Establish whether behavioral, cognitive, and emotional states affect immune system function by conducting careful, quantitative studies with sufficiently large numbers of subjects to yield clear-cut results.
- Identify behavioral, neuroendocrine, and immune pathways that link psychosocial factors to the onset and pathogenesis of infectious disease in humans.
- Explore behavioral conditioning and/or vaccinations as therapeutic modalities for treatment of behavioral dysfunctions.
- Discover novel mediators of mechanisms of neuro-endocrine-immune-behavior interactions.
- Identify the changes in the immune system occurring outside the central nervous system that impact on or affect behavioral states, and determine cellular and molecular mechanisms underlying these effects.
- Elucidate how immune tolerance is established and maintained in the brain as compared to other nonlymphoid tissues.
- Determine physiological, pathophysiological, environmental, and sensory factors that stimulate elaboration and release of molecules common to the central nervous and immune systems (e.g., cytokines) within the brain.
- Analyze common aspects of the nervous and immune systems' structure and function in general, and identify and functionally characterize common or related receptors and these receptors' antagonists, in particular.

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NERVOUS SYSTEM INTERACTIONS IN THE IMMUNE SYSTEM

"If you look at the totality of the way the system works, you have a constant conversation going on at every level within the body, and this conversation really forces you to think in a more integrated sense."

—Seymour Levine

The immune system, like the nervous system, was once thought to be a self-contained and self-regulated entity. As the evidence mounted that mood and stress can affect the ability of the immune system to ward off disease, researchers began looking for the pathways by which the central nervous system could influence the immune system. In searching for these links, investigators hope to find out how stress, depression, behavioral conditioning, and other psychological factors may be related to susceptibility or resistance to infectious and autoimmune diseases and how behavioral or neuropharmacological interventions might be used to either boost or attenuate the immune response.

Multiple Pathways From Brain to Immune System

So far, research has shown that the immune system reacts to powerful hormones released by the brain in response to stress. It is widely accepted that the hypothalamus releases the hormone called corticotropin-releasing factor, which travels to the anterior portion of the pituitary gland and causes it to secrete two other hormones, adrenocorticotropin (ACTH) and β-endorphin. ACTH acts on the adrenal gland to release hormones known as glucocorticoids.

Complex feedback loops connect the immune system and the brain.

Low levels of glucocorticoids are necessary for normal immune system function, and high levels have the opposite effect. Some of this may have to do with complex feedback loops. Cytokines, including various interleukins and tumor necrosis factors, and prostaglandins are part of the feedback loop stimulating corticotropin-releasing factor release by the hypothalamus. Each point in this loop—there are dozens, with more being discovered—may be vulnerable to disruption by infectious agents or other insults.

This is certainly one of the major routes by which the brain coordinates immune function. It is well established, for example, that removing the adrenal gland markedly reduces the effect of stress on certain parts of the immune system, providing strong support for the idea that steroid hormones made by the adrenal gland play a critical role in the connection between the nervous and immune systems.

Other pathways fine-tune the immune system.

However, other pathways also play a role in fine-tuning the immune system's reaction to various challenges. Some of these pathways may involve direct connections between nerves and the organs associated with the immune system, or even the immune cells themselves.

Multiple pathways may seem to be a waste of resources, but with the critical role that the nervous and immune systems play in maintaining homeostasis, such redundancy may be important for keeping both systems operating at optimal levels under a variety of ever-changing conditions. Working out these pathways is a critical challenge in the field of psychoneuroimmunology, one that investigators are now pursuing with all the tools at their command.

Direct Neural Connections in Lymphoid Organs

Investigators are now mapping neural circuits in the lymphoid organs. These studies are necessary to understand the link between behavior and immunity. Already, mapping efforts have found that neurons often follow pathways set down by blood vessels. Several such neural circuits, each using a different neurotransmitter, have been tracked into the spleen. Neural circuits in bone marrow may help immune cells make their way to various target organs, a phenomenon known as homing.

The nervous system probably sends suppressive signals to the spleen.

Administering a compound that blocks the sympathetic nervous system's ability to stimulate the spleen eliminates the detrimental effects of stress on lymphocytes in the spleen. The lymphocytes do not show suppressed activity under stress, presumably because the nervous system can no longer send some important suppressive signal to the spleen.

Neurons may directly stimulate developing lymphocytes in lymph nodes as well as in the spleen and thymus. Nerve terminals are found just a few millionths of an inch from both T-lymphocytes and macrophages, the smallest gap yet found between a nerve ending and its target cell. During development of the spleen, nerve terminals appear first in the regions where lymphocytes mature. Only later do they grow into the spleen's parenchyma. In fact, such innervation may play a role in the proper development of the spleen as a lymphoid organ.

Assuming that these nerves are targeting lymphocytes, researchers are now studying the effects on nerve endings of removing these cells as well as the effect on lymphocyte development of destroying the nerve terminals. Following treatment with a drug that eliminates lymphocytes from the spleen, the nerves seem to retract within the spleen, though they remain capable of transmitting nerve signals. However, using various antigens to challenge an animal with a depopulated spleen causes the nerves to degenerate. Further study has shown that this is probably a result of interleukin-1 somehow interacting with the nervous system.

Repeated challenges reduce the ability of the immune system to reac

This remarkably stable system, therefore, turns out to be stable in the face of loss of target cells but not so stable during an immune response, an effect that may be driven by cytokines. This seems to imply that the immune system might lose some of its ability to react to antigen challenge after repeatedly responding to such challenges. In fact, an experiment with animals supports this idea. One group of animals was constantly challenged with various antigens, while the other lived in a pristine environment. After 26 months, there were 50-percent fewer nerve endings in the spleen and thymus of the challenged animals than in the protected animals.

Investigators have also examined this effect in a strain of mouse that is born without T-lymphocytes and B-lymphocytes, though it has natural killer cells and macrophages. Surprisingly, the density of nerve endings in the spleen and thymus of these animals is normal. The reason may be that the macrophages are responsible for maintaining innervation in these lymphoid organs.

Autoimmune disease may be partly a neural phenomenon.

These findings may help researchers solve some critical questions concerning autoimmune diseases, such as arthritis. For example, animals with drug-induced arthritis and with the nerve terminals in their lymph nodes destroyed have far greater swelling and faster tissue degeneration than those animals with normal innervation of the lymph nodes. However, if only certain nerve terminals are destroyed—those that use the neuropeptide known as substance P—the animals fail to develop autoimmune arthritis. This suggests that susceptibility to autoimmune disease is not only an immune phenomenon, but is at least in part a neural one.

Neurally Derived Substances Affect Immune Cells

If the immune system is regulated by substances released from the nervous system—neurotransmitters or neurohormones—then there must be receptors for those neurally derived substances either on the surface or inside of immune cells. These receptors may be present at all times or they may appear only when the immune cells are activated by exposure to antigen, cytokine, or both. In all likelihood, the biochemical events triggered when a neurally derived substance binds to its receptor will not themselves activate an immune cell but will instead play a modulatory role in the development of a mature immune cell.

The hormones prolactin and growth hormone, for example, bind to receptors that belong to the same family as those to which many cytokines bind. In addition, the folding of growth hormone is similar to that of interleukin-2, even though the two molecules have different amino acid sequences (fig. 5). Both molecules have a bundle of four helices as a major structural motive. These similarities have prompted investigators to look at the possibility that two seemingly dissimilar molecules produced by different systems may be interacting with the same cell-surface receptors.

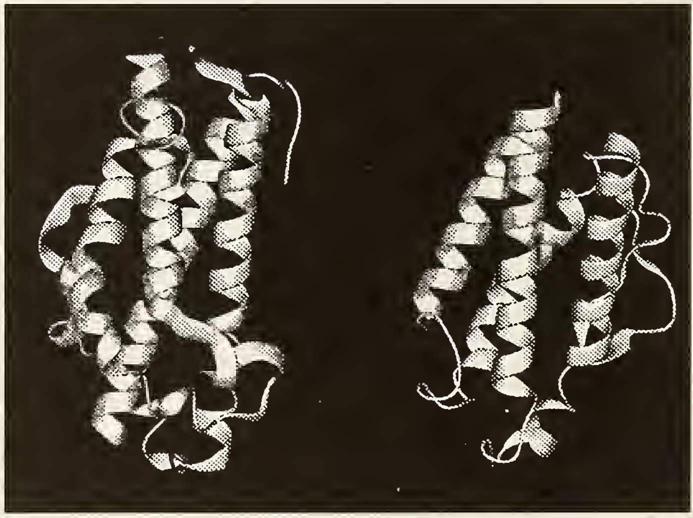

FIGURE 5.—The three-dimensional similarity between growth hormone (left) and cytokine interleukin-2 (right) is revealed by "ribbon diagrams" of these distinct molecules. These computer-generated images were created using Protein Data Base files (denoted 1HUV and 3INK) of the Brookhaven National Laboratory.

[Courtesy V. Novokhatny.]

Chaos in the immune system is reduced by using second messengers.

Such molecular cross-talk could lead to chaos in the immune system as a lymphocyte or macrophage struggles to decipher the wave of incoming chemical signals. But immune cells appear to have a sophisticated mechanism for using this information in directing their development. This mechanism, used throughout the nervous system, is called the second messenger system. When an extracellular molecular signal, be it a cytokine, neurotransmitter, neuropeptide, or hormone, binds to its receptor embedded within the cell wall, a cascade of molecular events begins that produces another molecule within the cell—the second messenger. This, in turn, triggers further events, such as a change in gene expression, enzyme activity, or ionic balance. Each receptor on a given cell type, though it binds a unique substance, is tied into a number of messenger systems, providing a means of integrating multiple incoming signals through the transduction mechanism of the receptor.

Lymphocytes may receive simultaneous signals from several sources.

The molecule cyclic-AMP (cAMP) is a common second messenger, and investigators have found that the timing of cAMP production within a lymphocyte is critical for activating that cell. The accumulation of cAMP in lymphocytes can occur through the activation of receptors on these cells by certain naturally occurring substances such as neurotransmitters called catecholamines. If cAMP production is increased in lymphocytes in the absence of immune signals, it is unlikely to have a profound or long-lasting effect. However, when cAMP production is increased at or about the same time as the lymphocyte receives an immune signal, lymphocyte function is greatly altered. Because it is difficult to predict when or where these two signals will reach the lymphocyte, spatial and temporal concerns become important. In the living body, lymphocytes may receive signals from two or more neurally derived substances as well as the immune signal, adding further complexity to how lymphocyte function is altered (fig. 6).

A New Mediator?

Recent experiments suggest that factors other than neurotransmitters and steroid hormones may be able to suppress immune system activity. One such candidate, still unidentified, is a large molecule present in the blood of stressed animals that can suppress the activity of lymphocytes removed from animals that had not been stressed. This suppressive factor also downregulated cytokine production by lymphocytes as well as *in vitro* activity of natural killer cells and was able to keep immune cell function suppressed even 24 hours after exposure. Thus, this soluble factor may play a role in forming what could be considered a molecular memory of stressful events, perhaps by blocking expression of interleukin receptors on the surface of lymphocytes.

What is certain is that there are multiple pathways by which the brain can signal—and affect—the immune response. Uncovering these pathways and determining their relevance to specific behavioral and immunological conditions is a daunting challenge. Surely, such redundancy has important biological consequences, and it will take a concerted effort by researchers in many fields to make sense of this complex communication system.

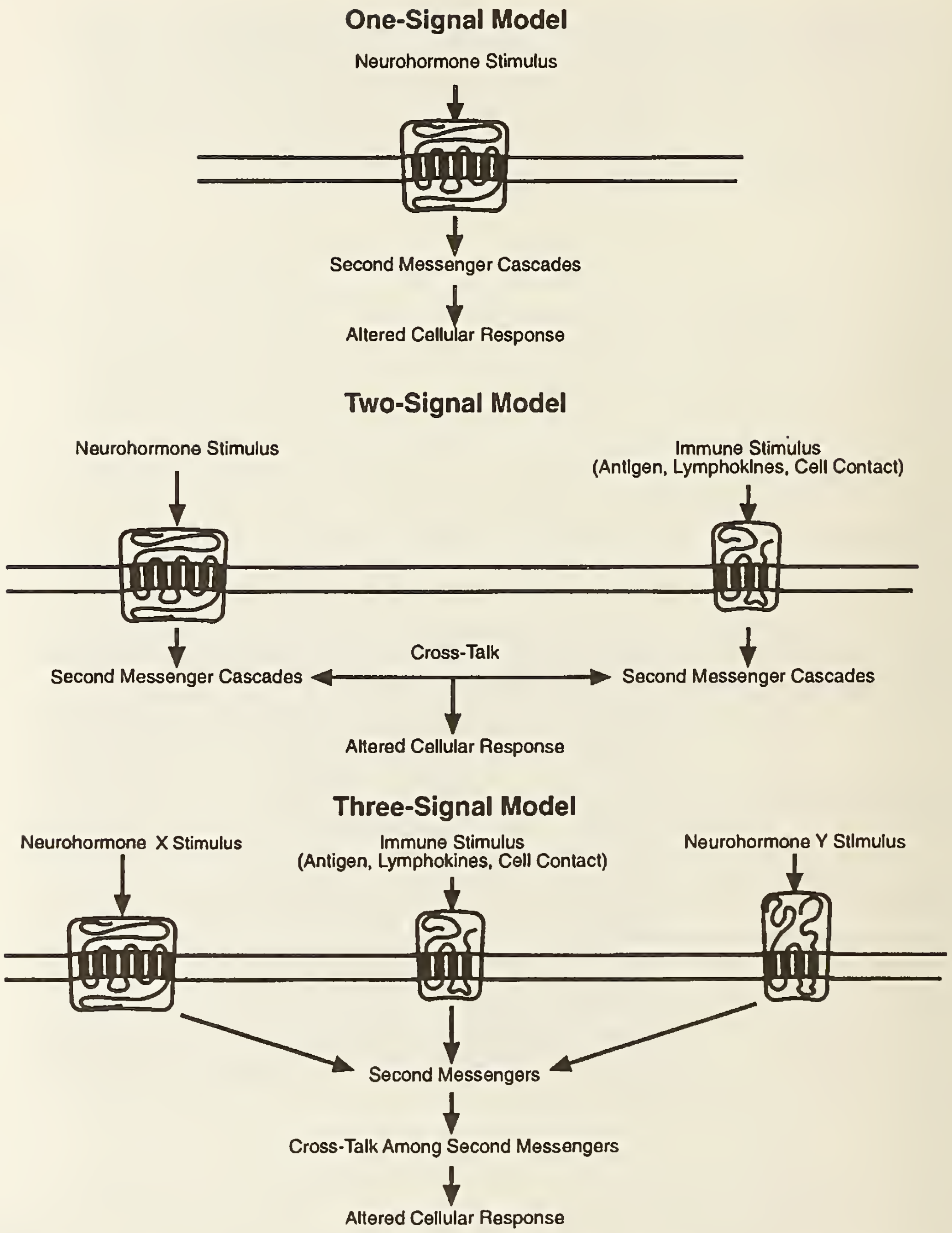
One-Signal Model
Neurohormone Stimulus
Second Messenger Cascades
Altered Cellular Response
Two-Signal Model
Neurohormone Stimulus
Immune Stimulus
(Antigen, Lymphokines, Cell Contact)
Cross-Talk
Second Messenger Cascades
Second Messenger Cascades
Altered Cellular Response
Three-Signal Model
Neurohormone X Stimulus
Immune Stimulus
(Antigen, Lymphokines, Cell Contact)
Neurohormone Y Stimulus
Second Messengers
Cross-Talk Among Second Messengers
Altered Cellular Response

FIGURE 6.—Models of one-, two-, and three-signal transduction mechanisms in lymphocytes. Note the increase in complexity with the increasing number of signals simultaneously affecting a single cell. The cell depicted here is a lymphocyte, but it could be any other type. Most cells, but particularly those in the brain, simultaneously respond to an unknown but certainly large number of signals. Signal receptors are predominantly proteins positioned in the cellular membrane, depicted here by a fence-like structure. [Courtesy T. Roszman.]

Recommendations for the Future

- Determine how neuroimmune interactions may be skewed in different individuals depending on their previous behavioral and immune experience.

- Identify synaptic interactions by which cortical limbic signals alter autonomic, endocrine, and behavioral responses that affect the immune system.

- Characterize functional changes occurring *in vivo* in immunocytes elicited by hormones and neurotransmitters.

- Elucidate how hormones, neurotransmitters, and neuropeptides affect immunocytes' functions at the molecular and cellular levels.

- Elucidate local effects on immune activity reflecting neurotransmitter usage in a particular location outside the central nervous system.

- Determine whether locally elaborated immune modulators affect the peripheral and central nervous system, producing a global feedback response.

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IMMUNE INTERACTIONS IN THE NERVOUS SYSTEM

"Four or five years ago, it was apparent that this system was complex. All I can say is, it's become infinitely more complex since then."

—Samuel M. McCann

The brain is the most privileged organ in the body. It takes precedence over other organs for nutrients and oxygen and is highly selective in what it allows to enter. Even substances circulating in the blood are prevented from reaching the brain by the blood-brain barrier, which permits only specific cells and molecules to cross over.

The immune system fights infection in the brain, as elsewhere.

Until recently, investigators thought that immune cells did not enter the brain. A healthy human brain has few signs of antibodies, lymphocytes, or any other immune system components. However, research has now shown that the immune system can mount a significant response to infection or damage in the brain. In addition, some substances released by the immune system to fight infection elsewhere in the body are known to enter the brain and may play one or more roles in the mood changes and other behavioral symptoms that commonly accompany illness.

It is also clear that the immune system can malfunction in the brain, just as it can in the rest of the body. The immune response can be inadequate, allowing invading organisms to damage brain cells or even use them as a reservoir for later release and infection. The immune system may also mistakenly attack healthy brain tissue.

Communication between the immune system and the brain is crucial for regulating the immune response throughout the body. Investigators have found that cytokines can stimulate the stress-response system in the brain and, conversely, that the hormones released in reaction to stress can suppress the immune system under some circumstances. Apparently, this interaction with the brain helps keep the immune system in check.

A Normal Immune Response

Activation of the immune system produces a host of physiological and psychological changes, including altered blood flow and hormone levels, a rise in body temperature, loss of appetite, and lethargy. One such change, fever, appears to result when neurons in the hypothalamus, the part of the brain that regulates body temperature, are activated by cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor-a and probably other molecules still to be discovered.

Interleukin-1, a cytokine, is an extremely potent neuroactive substance.

Experiments have shown that extremely low levels of both interleukin-1 and interleukin-6 can activate the fever response. Injecting an exceedingly tiny amount (0.6 picomoles) of interleukin-1 directly into the brain triggers a fever; few if any other neuroactive substances are this potent. The same effect is seen when a fever-inducing component of bacterial cell walls, known to stimulate interleukin-1 release, is injected into brain.

The levels of stress-response hormones also increase in the bloodstream following administration of a small quantity of either interleukin-1 or the bacterial cell wall component. The amount of corticotropin-releasing factor, which triggers the body's response to stress, traveling from the hypothalamus to the pituitary rises significantly. This leads to an increase in the release of other stress–response hormones such as cortisol and ACTH.

Interestingly, elevated levels of corticotropin-releasing factor are found in the extracellular fluid of the brain in depressed individuals. When rats are subjected to stress, this hormone is concentrated particularly in the region of the brain called the locus ceruleus, where changes in neuronal activity are associated with depression. These observations suggest that corticotropin-releasing factor plays a role in depression precipitated by stressful conditions. The precise regions of the brain where corticotropin-releasing factor exerts its effect in depression are presently under investigation.

Cytokines in the Brain

Investigators now believe that cytokines from outside the brain act on the brain at certain "windows," places where these molecules have access to the central nervous system. These windows are thought to be at sites along the edges of the ventricular system that lack a blood-brain barrier and where neurons have the opportunity to respond to cytokines circulating in the bloodstream. One such window lies next to the hypothalamus, though it is far from certain that this site plays any role in the ability of cytokines, particularly interleukin-1, to produce fever. However, the discovery of compounds that can block interleukin-1 by binding to its receptor should make it possible to test this hypothesis.

Cytokines may act directly on neurons, much as if these substances were neuropeptides. In fact, antibodies specific for interleukin-1 bind to neurons in the hypothalamus and brain stem, indicating that this cytokine is present in those neurons. At least some of these neurons project to hypothalamic cells that produce corticotropin–releasing factor. Studies in mice have found another cytokine, tumor necrosis factor-α, in many of the same neurons, particularly those in the hypothalamus.

Cytokines may act directly on neurons.

In all likelihood, researchers will find that interleukin-1 is produced in many regions of the brain and acts by mechanisms specific to each region. In the hypothalamus, for example, interleukin-1 appears to act on neurons that release corticotropin-releasing factor. Studies have shown that interleukin-1 turns on the genes that code for corticotropin-releasing factor. This effect is eliminated if corticotropin-releasing factor antiserum—antibodies that bind to the hormone—is administered prior to interleukin-1. Presumably, interleukin-1 still induces the hypothalamus to produce and release corticotropin-releasing factor, but the antibodies bind to and neutralize this hormone as soon as it is released into the blood stream.

Interleukin-1 probably affects different regions of the brain differently.

This antiserum also blocks the effects of the cytokines interleukin-6 and tumor necrosis factor-α, suggesting that interleukin-1 activation of corticotropin-releasing factor release is a necessary first step in any cytokine-induced activation of the stress-response system. The effect of this activation, if strong enough, may be to downregulate an ongoing immune response. Within 15 minutes after interleukin-1 is administered in the brain, a number of immune system cells outside of the brain show marked decreases in activity.

This downregulation may play a role in the immune suppression characteristic of AIDS. Studies have shown that infusing the protein gp120, found on the surface of HIV, into the brain stimulates interleukin-1 release in brain tissues. It also provokes a rise in plasma steroids—part of the stress-response system—and a decrease in natural killer cell activity. Injecting compounds known to interfere with interleukin-1 activity blocks these effects.

Immune suppression, as in AIDS, may be related to interleukin-1 activity in the brain.

One of the most potent inhibitors of interleukin-1 is actually produced in the brain—a peptide called melanocyte-stimulating hormone. On a molecule-by-molecule basis, melanocyte-stimulating hormone is 25,000 times more potent for reducing fever than aspirin, a well-known fever reducer, presumably by blocking the effects of interleukin-1 in the hypothalamus. In fact, a number of studies have now shown that administering this hormone blocks the release of corticotropin-releasing factor from the hypothalamus. At least some investigators now believe melanocyte-stimulating hormone could prove therapeutically valuable in eliminating the effects of excessive production of interleukin-1 and cytokines with similar action, such as interleukin-6 and tumor necrosis factor-α.

Alternative Pathways

Though it is still unclear how the interleukins interact with neurons in the hypothalamus, neuroscientists have found that prostaglandins play a key role in the process. Prostaglandins are involved in many important biochemical processes, at least some of which are inhibited by aspirin. Prostaglandins are made from the lipid arachidonic acid via a long series of chemical reactions. Blocking a key enzyme in this series of reactions blocks the release of corticotropin-releasing factor.

Prostaglandins, key to interleukin-neuron interaction, are made by neurons.

A search for prostaglandins and this key enzyme in brain tissue revealed some surprises. One was that only neurons, and apparently not glia, produce prostaglandins. Another interesting finding was that the enzyme was more prevalent in areas of the brain associated with higher cognitive functions, including the hippocampus and limbic system (which plays a role in controlling mood and affect), than in lower brain centers.

Interleukin-1 may also work by stimulating the release of the hormone vasopressin. This idea is supported by experiments showing an increase in the expression of vasopressin messenger ribonucleic acid (mRNA) levels—a measure of gene activation—following interleukin-1 administration into the brain. In addition, injecting either antibodies against vasopressin or vasopressin antagonists prior to interleukin-1 blunts the stimulatory effect that interleukin-1 (injected into brain ventricles) has on the pituitary gland's secretion of the stress hormone ACTH. Thus, it appears that vasopressin may represent a second pathway by which interleukin-1 activates the release of ACTH.

Nitric oxide is an important modulator of many biochemical processes.

A new element in this picture is nitric oxide, which only recently was found to be an important modulator of many biochemical processes, including gene expression in neurons. (Nitric oxide is different from nitrous oxide, the chemical commonly used as an anesthetic.) A number of studies have shown that interleukin-1 stimulates the release of nitric oxide by turning on production of an inducible form of the enzyme that synthesizes nitric oxide, so-called inducible nitric oxide synthase (iNOS). A more recent experiment found, though, that blocking nitric oxide release boosted the ability of blood-borne interleukin-1 to increase ACTH secretion. This augmenting effect, interestingly enough, is seen with one other stimulus—cocaine. The implications of these results are presently unclear, but they are providing researchers with another lead for studying the link between the immune system and the brain.

Immunoprotection in the Brain

Much is still unknown about what happens when the central nervous system itself is infected or damaged. Instead of thinking of the brain as immunologically privileged, researchers today find it more useful to consider the brain as being immunologically quiescent, or downregulated, under normal circumstances and immunologically active, or upregulated, in disease states. This is not unique to the brain, for the same holds true for most other organs in the body. Healthy muscle, for example, is not infused with lymphocytes either.

The brain is immunologically quiescent under normal circumstances.

Under normal conditions, lymphocytes and other immune system cells circulate (traffic) through the brain and spinal fluid and back out to the periphery, that is, outside the central nervous system, seeking any foreign antigens or signals indicating significant cellular abnormalities. When necessary, immunocyte trafficking increases in frequency. There is evidence that stimulated lymphocytes secrete enzymes that enable them to cross the blood-brain barrier, just as they can cross through other tissues elsewhere in the body.

It also appears that chemical attraction signals, released from still-unknown sources, somehow direct lymphocyte movement into, within, and out of the brain. Some of these signals may involve cytokines. Other molecules, such as integrins and other cell-adhesion molecules, that are expressed on the surfaces of cells may also play a role in bringing lymphocytes into the brain. Understanding this interface between the brain and the immune system represents a major research challenge and is an important priority among neuroscientists and immunologists.

Chemical signals from unknown sources attract lymphocytes to the brain

Another unknown is the role that binding proteins play in helping cytokines enter the brain and reach their targets. These cytokine-binding proteins normally render cytokines inactive, but when released from these carriers—the triggers are unknown—the cytokines can activate a host of biological actions.

In recent years, investigators have learned that microglia and astrocytes can release cytokines to stimulate an immune response. These cells may also display on their surfaces the same antigen-presenting MHC molecules that immune system cells express to signal the presence of an invader.

Interest in the contribution of astrocytes to immunological abnormalities in the central nervous system came about from studies of multiple sclerosis, which is a central nervous system disease of unknown etiology, though it certainly has an immunological component. In multiple sclerosis, the number of T-lymphocytes and the amount of various cytokines in the central nervous system show a noticeable increase. The number of cells expressing major MHC class-II molecules, which target cells for attack by T-lymphocytes, also increases. Researchers studying multiple sclerosis thus focused on astrocytes surrounding brain lesions in the patients. These astrocytes, when stimulated, express MHC class-II antigens and produce a wide array of cytokines, including interleukin-1, tumor necrosis factor-α, and interleukin-6.

While cells in the central nervous system do not normally express MHC class-II antigens, experiments in animals have shown that the cytokine γ-interferon will stimulate such expression. Certain viruses induce the same result. This expression is enhanced, but not turned on, by tumor necrosis factor-α. How this happens is still unclear, though it may be that tumor necrosis factor promotes more efficient expression of the MHC class-II antigen genes turned on by γ-interferon.

Glia can express MHC class-II antigens

Using cultured astrocytes, investigators have learned that MHC class-II expression is induced by γ-interferon and certain viruses. Tumor necrosis factor-α has no effect on MHC class-II expression but can synergize with either γ-interferon or virus for enhanced MHC class-II expression. The researchers have also found that MHC class-II expression is more easily downregulated on astrocytes than on microglia, implying that different modes of regulation occur in these two cell types.

Three regulatory elements (boxes), W, X, and Y, in MHC class-II genes have been studied. These elements are important for constitutive expression (i.e., always expressed) of the gene in some cells, such as B-cells and macrophages, and inducible expression (i.e., only expressed when stimulated by some factor) in other cell types. To further probe this system, investigators took the portion of the MHC class-II gene containing the W, X, and Y boxes, linked it to a reporter gene, and inserted it into rat astrocytes. If the boxes were functional, the reporter gene product would be made and would be identifiable after treating the astrocytes with γ-interferon.

To probe the regulatory elements, the investigators mutated specific conserved regions (i.e., sequences in the gene that are the same across species) in the W, X, and Y boxes. Key mutations in any of these three regions knock out inducibility by γ-interferon. The investigators then looked for the protein in the cell nucleus that might be binding to these regions and mediating the induction; they found a protein that bound specifically to the X box. Mutations in the X box prevented the protein binding. Mutations in the W and Y box did not prevent this protein from binding to the X box, but blocked the ability of tumor necrosis factor-α to enhance γ-interferon-mediated induction of MHC class II.

Based on these studies, the researchers proposed that γ–interferon alters the binding ability of X-binding protein, perhaps by triggering enzymatic phosphorylation of the protein. Modifying the protein somehow turns on the MHC class-II gene. Tumor necrosis factor-α may augment whatever changes in the X-binding protein are triggered by γ-interferon.

Interestingly, similar experiments conducted in rat microglial cells reveal the presence of γ–interferon-induced X-binding protein; however, tumor necrosis factor-α does not amplify expression in these cells. These results emphasize that expression of MHC class-II genes is differentially regulated in astrocytes and microglia.

Astrocytic and microglial expressions of MHC class-II genes are regulated differently.

Investigators are keenly interested in finding the biological stimuli that induce MHC class-II genes in the nervous system, because glial cells expressing these genes may play a critical role in triggering an immune response within the central nervous system. The X-binding protein may represent a new class of transcription factor, which may give investigators an insight into the earliest steps of immune responses by glial cells.

A Host of Possibilities

Clearly, researchers are just beginning to touch on some of the important questions about the actions of the immune system in the brain. The exquisite potency of interleukin-1 in the brain, for instance, suggests that this cytokine may, in fact, be a neuromodulator, a compound that attenuates a nerve's response to a neurotransmitter. Indeed, it brings into question whether cytokines and neuromodulators are really one and the same—compounds whose functions are determined by the environment in which they are released.

Multiple systems may be controlling the same effect.

Current research also raises the issue of redundancy, that is, whether multiple systems might be controlling the same end effect. This idea is supported by the finding that interleukin-1 seems to act in the brain through at least two intracellular mediators. On one level, interleukin-1 can induce a cytokine cascade in glial cells. At another level, interleukin-1 triggers production of signaling molecules and intracellular mediators. Further work is needed to tease out the nature of this redundancy and to understand how different systems, if they do exist, might work together to regulate both immune responses and behavior.

Recommendations for the Future

- Elucidate mechanisms of immunocyte trafficking through the central nervous system and develop ways to alter this trafficking.

- Determine whether immune cells function in qualitatively different ways in the brain than in other nonlymphoid organs.

- Determine exogenous cellular (e.g., pathogens, tumors) and molecular (e.g., psychoactive drugs) factors that enhance/inhibit passage of immunocytes into the central nervous system.

- Elucidate immune reactions in the brain directed at answering the following questions:
  - How do immune effector cells and molecules gain access to their neuronal target antigens?
  - Which subcellular compartments in a neuron are accessed by an antibody, and what are the effects of the antibody's entry into these compartments on neuronal function?
  - What effector mechanisms can protect neurons from viral infection?

- Analyze the antigen-presenting potential of endogenous brain cells other than neurons, how antigen presentation is regulated in the brain, and how antigen-presenting cells in the brain compare with those of other tissues.

- Determine mechanisms by which immune stimuli (e.g., cytokines) act on the brain to cause changes in behavior and in autonomic and endocrine responses.

- Analyze the effects of microglial and macroglial secretory factors known to be capable of affecting both immunocytes and neurons.

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AUTOIMMUNITY AND NEUROIMMUNOLOGICAL DISEASES

"Autoreactivity does not always mean pathology."

—Keith A. Krolick

Recent research has raised the possibility that some autoimmune diseases may be as much a neural as an immune problem, opening new avenues of inquiry that may lead to novel therapeutic approaches to these devastating diseases. In similar fashion, investigators are learning more about the immunological mechanisms that are involved in various central nervous system diseases, such as multiple sclerosis and Alzheimer's disease, again raising the possibility of clinical breakthroughs in the not too distant future.

Some immune system cells are not exposed to "self" molecules during their development.

Normally, the immune system is programmed not to recognize self molecules that are present in or circulate through the thymus. Any self-responsive T or B cells that come into contact with their specific target antigens are programmed to die. However, some autoreactive immune cells are not exposed to their specific self molecules during development, especially to organ-specific antigens such as those found in the central nervous system, peripheral nervous system, connective tissue, and pancreas. These autoreactive cells are not removed from circulation and are present at low levels even in normal healthy individuals.

Under abnormal conditions, for example, when viral infections induce inflammation and damage the organ, the number of autoreactive cells may be selectively expanded. When these cells reach a critical threshhold level, they may attack the self antigen in the tissue, leading to autoimmune diseases such as multiple sclerosis, Guillan-Barre syndrome, rheumatoid arthritis, and diabetes.

Alternatively, an invading foreign antigen may possess a particular molecular moiety that "looks like" part of a self protein to the immune system. This is the concept of "molecular mimicry." Thus, in attacking the invader, the immune system may mistake self for non-self.

Multiple Sclerosis

One of the first brain diseases thought to be caused by an autoimmune response, multiple sclerosis (MS), manifests itself as impaired sensory or motor performance; this latter defect occurs infrequently. MS is thought to result from altered neurotransmission caused by T-lymphocytes specific for myelin antigens. Thus, T cells specific for basic protein (BP) or proteolipid protein (PLP) may cross the blood-brain barrier to produce central nervous system inflammation and destruction of the myelin sheath that wraps axons. Oligodendrocytes, which lay down the myelin sheath, are initially spared but may eventually be destroyed, thus limiting myelin regeneration. In most patients, MS is progressive, although it is common for symptoms to improve spontaneously and then later reappear and worsen.

An Animal Model

An animal model is helping to elucidate the causes and pathogenesis of multiple sclerosis.

An autoimmune disease similar to MS, called experimental auto–immune encephalomyelitis (EAE), can be induced in laboratory animals by injecting BP or PLP with adjuvants that enhance immunization and increase the number of myelin-specific T cells. Within 2 weeks, the animals develop progressive paralysis and some demyelination. The extent of demyelination varies with the species of animal and occurs predominantly in the central nervous system. Thus, EAE has become an important animal model for multiple sclerosis.

T cells that cause EAE tend to use receptors very similar to those involved in MS. By immunizing animals with a peptide representing a conserved region of the pathogenic T-cell receptor (TCR), it has been possible to induce TCR-specific T cells and antibodies that can neutralize the harmful T cells and prevent later induction of EAE. Perhaps more importantly, as least concerning possible clinical applications, immunizing animals that already have EAE with this TCR fragment stops the disease process. This therapeutic effect is rapid and dramatic, causing a decreased number of T-lymphocytes that can recognize basic protein. More work is needed to clarify the role of TCR in EAE. This work is progressing along well-defined lines, as outlined in table 1.

Another approach to prevention and treatment of EAE involves oral administration of the basic protein. Investigators have shown that orally administering BP to rats or mice before or at the first sign of clinical disease suppresses clinical symptoms. The mechanism underlying oral tolerance has been shown to be clonal anergy of

Table 1.—Role of T cell and its receptor in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis

What We Know

1. T cell-receptor (TCR) peptides prevent and alleviate experimental autoimmune encephalomyelitis.
2. Effects are mediated by anti-TCR peptide-specific T cells and antibodies.
3. Effective TCR protein sequences represent natural idiotopes.
4. Treatment with TCR pepides decreases functional response to the basic protein but does not delete a subset of T cells, called Vγ8.2+ T cells, from the outside or within the central nervous system, suggesting anergy (diminished reactivity to specific antigens).
5. The TCR/anti-TCR regulatory network is independent from and can be superimposed upon natural recovery mechanism(s).

What We Do Not Know

1. Why is neonatal tolerance to TCR idiotopes not induced *in vivo*?
2. How does natural immunization to TCR sequences take place *in vivo*?
3. Is cell-cell contact required for regulation to occur, or can soluble cytokines mediate the effects?
4. Are other cell types (e.g., CD8+ T cells) involved in regulation?
5. How and where does regulation occur, and what is the fate of the regulated T cells?

Future Directions

1. Identify functional defects in anergized T cells.
2. Generalize and optimize anergy induction in specific T-cell subsets.
3. Develop approaches for depletion of autoreactive T cells.
4. Develop strategies for identifying autoreactive pathogenic T-cell specificities.

[After H. Offner]

BP-specific T cells or suppression of specific T cells through elaboration of suppressive cytokines.

Restraint stress suppresses EAE in animals.

EAE can also be suppressed in rats and mice when animals are exposed to restraint stress. Such stress, which produces elevated levels of adrenal glucocortocoids and catecholamines, results in decreased clinical signs and depressed systemic immune responses to neuroantigens.

T-Lymphocyte Response in Humans

While TCR peptide therapy in animals offers hope for clinical advances, two important questions must be addressed in order to apply this strategy to MS: Is there evidence for T-lymphocyte response to myelin antigens in multiple sclerosis patients? If so, will immunization with the receptor fragments boost the immune system's ability to prevent T-lymphocytes from attacking myelin?

Longitudinal analyses revealed that MS patients had an elevated frequency of T cells specific for BP, both in the blood and the cerebrospinal fluid. Like MS itself, the T-cell response was episodic and reached levels similar to those found in animals with EAE. Moreover, as in EAE, the T cells tended to utilize common TCR molecules. A conserved region was identified from two such TCR sequences, and corresponding peptides were synthesized for use in a clinical trial.

Encouraging responses to treatment have prompted further clinical studies.

As in EAE, injection of the peptides successfully boosted TCR-specific T cells in a majority of the progressive MS patients tested, and in some patients, the response to BP was reduced. The effect was selective, in that responses to other microbial antigens were not affected, and no toxicity could be detected from the treatment. These encouraging results have prompted further studies in a larger group of MS patients to assess clinical efficacy.

A small clinical trial was also conducted testing the efficacy of administering myelin orally to patients with relapsing-remitting multiple sclerosis. Those individuals receiving oral myelin showed fewer declines in performance than those receiving placebo (an inactive pill made to look like an experimental drug). Interestingly, male patients showed greater clinical improvement than female patients. A larger trial is planned.

At the same time, researchers are still looking for the molecular trigger that induces such myelin-reactive T-lymphocytes. Unlike the situation in EAE, where encephalitogenic lymphocytes form in response to immunization with BP, some other event, for example, an infectious organism, must trigger the disease process in MS patients. Once a causative agent can be identified, it will be possible to target the organism and prevent MS. Meanwhile, the TCR peptide strategy, as well as other antigen-specific tolerance protocols, may provide selective regulation of autoreactive T cells to inhibit the MS disease progression.

Alzheimer's Disease: An Overaggressive Immune Response?

Alzheimer's disease is an age-related, progressive neurodegenerative disorder that has become increasingly identified with inflammatory mechanisms. Although there is no clinical test for Alzheimer's brain, samples collected at autopsy reveal several hallmarks of pathology that provide a definitive diagnosis. These include profuse fibrillar deposits of a molecule called amyloid β peptide and widespread formation of abnormal filaments (neurofibrillary tangles) in neurons. There is also substantial loss of neurons and their connections. These phenomena particularly occur in brain areas thought to subserve higher cognitive functions such as memory.

Evidence has accumulated over the last decade that inflammatory mechanisms may play a significant role in the brain damage characteristic of Alzheimer's disease. Initially, research on inflammation and Alzheimer's disease concentrated mainly on cataloging inflammatory markers and cells present in brain samples of affected patients. Among the most important of these were cytokines, such as interleukin-1, and complement proteins, a group of some 20 or more proteins that interact in a cascade to damage or destroy cells. The complement proteins were readily detectable in Alzheimer's brain samples and absent or greatly reduced in brain tissues from normal elderly people. They also appeared to be most prominent in those regions of Alzheimer's brain where damage to nerve cells occurs—the higher cognitive centers.

Inflammation occurs in brains of patients with Alzheimer's disease.

Subsequent studies have begun to elucidate mechanisms by which these processes might arise. In some respects, there are clear parallels to immunologic activity in other parts of the body and other human disorders. For example, virtually all the complement proteins have been found in Alzheimer's brain tissue, as have complement regulatory proteins, such as membrane inhibitor of reactive lysis and sulfated glycoprotein 2. The latter are upregulated outside the central nervous system as a means of controlling ongoing inflammatory attacks. Their presence in the Alzheimer's brain, and not in samples from normal elderly controls, strongly suggests that a functionally relevant inflammatory attack is ongoing in Alzheimer's disease.

In other respects, the immune activity in Alzheimer's disease may differ significantly from that in other organs and other disease states. Edema and neutrophil invasion, which are hallmarks of inflammation outside the brain, are not present in Alzheimer's. The activation of complement also appears to have an unusual source. Classically, complement reactions are initiated by antibodies. In Alzheimer's brain, amyloid β peptide appears to activate complement, as has been shown *in vitro* and confirmed in several independent laboratories. This, plus the continuing difficulty in unequivocally demonstrating brain-reactive antibodies in Alzheimer's patients, suggests that Alzheimer's may be an inflammatory disorder but not an autoimmune disorder.

Inflammation is an inherently destructive process. This would be particularly true in the brain, an organ that is heavily invested in neurons, a cell type that cannot be replaced, as many cells in other organ systems can. Inflammation may arise in the Alzheimer's brain as a response to previous damage. Once initiated, however, there is a high potential for a vicious cycle in which inflammation breeds more damage which breeds more inflammation. Whether as a primary event or not, the great weight of accumulated evidence strongly suggests that inflammation becomes a cause of damage in Alzheimer's disease.

Microglial activiation may prove relevant to AIDS dementia.

If nothing else, it should be clear from Alzheimer's research that a better understanding of immunologic mechanisms in human neural diseases may be expected to bear fruit. If inflammation can arise in Alzheimer's, then it is likely that it can arise under other conditions. Microglial activation, for example, is rampant in Alzheimer's disease, a phenomenon that may prove relevant to AIDS dementia.

Clinically, several retrospective studies have suggested an inverse relationship between Alzheimer's disease and a prior history of anti-inflammatory drug use. Perhaps the most compelling of these reports is a Duke University study of identical twins, one of whom had Alzheimer's and the other either did not or developed it significantly later. The twins were found to be significantly different on prior use of anti-inflammatory drugs.

Anti-inflammatory drugs may alleviate Alzheimer's disease.

Finally, a recent clinical trial was performed on Alzheimer's patients with encouraging results. Although very small, the study found that, on average, patients taking a placebo declined almost 10 percent on memory tests during the trial period. By contrast, patients taking an anti-inflammatory drug, indomethacin, that crosses the blood-brain barrier did not deteriorate and, in fact, showed nearly 2-percent improvement.

Perhaps because of the outworn dogma of the immunologically privileged brain, neuroimmunology may have become best known for showing how the nervous system affects the immune system. Like almost all relationships in the body, however, brain/immune interactions are probably a two-way street. Alzheimer's disease and multiple sclerosis make this abundantly clear.

Stiff-Man Syndrome

Stiff-man syndrome (SMS) is a rare disease of the nervous system characterized by progressive muscular rigidity and painful spasms. The symptoms result from the simultaneous activation of agonist and antagonist muscles, which, in turn, is the consequence of the continuous firing of α-motor neurons. Symptoms are partially improved by drugs that potentiate the action of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), such as benzodiazepines and baclofen. For this reason, it is believed that GABA-ergic neurons that are involved in the control of α-motor neuron activity are somehow affected in this syndrome.

Investigators studying a patient who had both SMS and insulin–dependent diabetes mellitus found that this patient's cerebrospinal fluid and serum contained autoantibodies directed against the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD). This enzyme is localized in neurons around the tiny sacs called synaptic vesicles that store GABA for release when needed.

SMS and insulin-dependent diabetes both involve anti-GAD autoantibodies.

Further studies of more than 100 patients with SMS established that more than 50 percent of them had anti-GAD autoantibodies. In addition, many of these SMS patients had insulin-dependent diabetes mellitus, a well-established autoimmune disease. Interestingly, both GAD and GABA are present in pancreatic cells that secrete insulin and are destroyed in the autoimmune attack leading to diabetes.

These findings raised the possibility that autoimmunity directed against GAD may have some role in insulin-dependent diabetes mellitus. Subsequent studies of insulin-dependent diabetes mellitus patients without SMS demonstrated that the large majority of these patients had autoantibodies directed against GAD and that GAD is identical to the so-called 64 kD autoantigen of insulin-dependent diabetes mellitus, a previously identified dominant antigen of diabetes.

Different types of autoimmune responses to GAD may correlate with different clinical conditions.

Although the autoantibodies found in SMS and insulin-dependent diabetes mellitus are similar, differences show up in certain analytical tests. Furthermore, the titre of anti-GAD autoantibodies in SMS patients is much higher than in insulin-dependent diabetes mellitus patients, suggesting that different types of autoimmune response to GAD may correlate with different clinical conditions. To date, investigators do not know the role of these autoantibodies in producing the neurological symptoms of SMS.

Not all who have SMS develop this specific antibody, and researchers have been examining this smaller group of patients as well. A few of these patients, all women, had autoantibodies against another neuronal protein called amphiphysin. This protein, like GAD, is localized in proximity to neuronal synaptic vesicles as well as other areas. Strikingly, all these SMS patients also suffered from breast cancer. In two cases, the search for (and detection of) an occult breast cancer was triggered by the previous identification of the antiamphiphysin autoantibodies. Removal of the tumors was always followed by an improvement in the neurological symptoms.

One neurological condition is related to two distinct sets of autoantibodies.

Again, it is not clear how antiamphiphysin autoantibodies are involved in producing muscle rigidity, and researchers are intrigued by the fact that the same neurological condition is related to two distinct sets of autoantibodies. Yet the two autoantigens share the property of being associated with synaptic vesicles. One possibility is that autoimmunity to both GAD and amphiphysin may somehow impair the efficiency of neurotransmitter release from synaptic vesicles.

“Autoreactivity Does Not Always Mean Pathology”

Autoreactivity may appear to be a universally pathogenic event, and immunologists once held that idea as fact. But in recent years, it has become clear that autoreactive antibodies are present in nearly everyone, and these antibodies do not cause any apparent harm.

This idea led researchers studying the neuromuscular disorder myasthenia gravis to look more closely at the autoantibodies against the acetylcholine receptor that characterize this disease. In myasthenia gravis, antibodies against this receptor interfere with the ability of acetylcholine to act as a neurotransmitter at the junction between motor neurons and muscle cells. These antibodies can, in some instances, also trigger the complement reaction, which attracts macrophages and other immune cells to the neuromuscular junction, exacerbating damage.

One observation that baffled clinicians is that the measurable levels of acetylcholine receptor antibodies do not correlate with disease severity in myasthenia gravis patients. In other words, some myasthenia gravis patients with high antibody levels have exceedingly mild symptoms, while some patients who have died from the disease have marginally detectable antibody levels. This has led investigators to propose that not all antireceptor antibodies are equally capable of interfering with acetylcholine receptor-dependent neuromuscular function.

Concentration of acetylcholine receptor antibodies does not correlate with disease severity in myasthenia gravis patients.

A corollary to this hypothesis is that antibody levels and disease severity will only correlate when the disease-causing antibody subsets approach 100 percent of the total antireceptor antibody level. A second corollary is that disease severity will not be determined by total antireceptor antibody levels, but rather by the level of a specific subset of autoantibodies that are able to interfere with neuromuscular function. Furthermore, it should be possible to identify those autoantibodies that are pathogenic using an *in vitro* assay of motor neuron function; only those autoantibodies that are pathogenic will block nerve signal transmission between neuron and muscle cell.

Immunizing an animal with acetylcholine receptor fragments causes B-lymphocytes to generate autoantibodies against the receptor and symptoms of myasthenia gravis to develop. Investigators are now using this model to identify the pathogenic antibodies and determine how they interact with the receptor. It appears that between 10 and 20 different antibodies react with the receptor, only some of which, as expected, are capable of producing disease symptoms when injected into healthy animals. All antibodies that are pathogenic could bind to the receptor in intact cells.

An animal model is helping identify pathogenic antibodies and their interactions with the acetylcholine receptor.

Now that they have identified the pathogenic autoantibodies, investigators are trying to determine what molecular event triggers their formation. Others, meanwhile, are looking at antibody heterogeneity in a variety of autoimmune diseases, trying to determine the role that both pathogenic and nonpathogenic autoantibodies play in the immune system's ability to function properly.

Studying Neuronal Death

Little is known at present about the mechanisms that trigger the nerve degeneration seen in disorders such as Parkinson's disease and amyotrophic lateral sclerosis. What is characteristic of all of these illnesses is that neurons in specific anatomical and functional groups die off with no evidence of prior pathology, and the disease appears secondary to the loss of these neurons.

One way to study neuronal death is to use neurotoxic drugs. One such drug, guanethidine, induces the destruction of sympathetic neurons in the superior cervical ganglion (SCG) that innervate organs physically above the diaphragm, including the thymus. One interesting property of guanethidine-induced cell death is that it happens quickly, within 5 days. Moreover, it is associated with chronic inflammatory response in the SCG. This is too fast for the typical T-lymphocyte-driven process that characterizes most chronic inflammatory processes.

Natural killer cells may kill neurons.

This observation led researchers to look for a different mechanism, one involving some other immune component. The search was fruitful, leading to the discovery that natural killer cells were responsible for guanethidine-induced neuronal death (fig. 7). Never before had researchers found an instance in which natural killer cells functioned as the primary effector cell in a neurodegenerative condition. Eliminating natural killer cells with an antibody that specifically binds these cells totally prevents guanethidine-induced neuronal death.

Having shown that natural killer cells can kill neurons directly in laboratory animals, investigators wondered if the same thing might hold true in some neurodegenerative diseases. Guanethidine and its

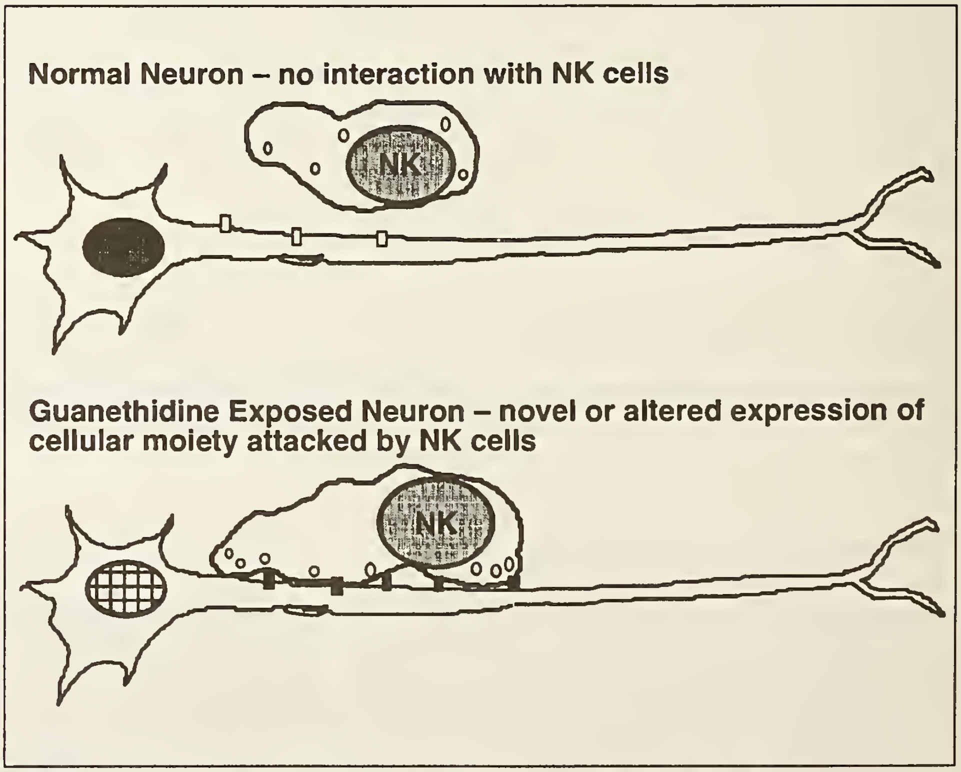
Normal Neuron – no interaction with NK cells
NK
Guanethidine Exposed Neuron – novel or altered expression of cellular moiety attacked by NK cells
NK

FIGURE 7.—Neuronal degeneration mediated by natural killer (NK) cells.

close chemical relative guanacline were once used as drugs for treating hypertension, so investigators poured through the medical literature to see if there were any reports of neuronal death in patients receiving these drugs. Sure enough, there were scattered cases of unremitting orthostatic hypotension in a small group of patients who had received therapeutic doses of guanacline. It may be that neuronal death was responsible for this irreversible side effect.

This has led investigators to propose a new mechanism by which neurodegenerative disorders might occur. Under normal conditions, natural killer cells and neurons coexist peacefully. At some point, though, a group of neurons is exposed to a toxin, be it a particular drug or some substance in the natural environment. This substance in and of itself is not toxic, and it would not affect most of the people who encounter it. But in a subset of individuals, this toxin finds its way into neurons and in some fashion alters the molecular composition of the neuronal membrane, making it "visible" to natural killer cells and triggering an attack that leads to neuronal death.

Normally, natural killer cells and neurons coexist peacefully.

An interesting sidelight to this research is that researchers do not know what natural killer cells actually recognize or fail to recognize. This research may open the door to finally identifying the targets of these key immunocytes.

Cytotoxic T-Lymphocytes

One subset of T-lymphocytes, called cytotoxic T-lymphocytes, is capable of suppressing viral replication by killing the cells that play host to the virus. Recently, investigators have found that this usually protective mechanism can have a pathological side to it. For example, disease symptoms might develop if the cytotoxic T-lymphocytes, in killing infected cells, also damaged healthy cells that happen to share some recognition molecule on their cell surfaces with the infected cells. Cytokines released by the cytotoxic T-lymphocytes might also produce pathological consequences.

Immune cells can become pathogenic.

Tropical spastic paraparesis is a neurodegenerative disease that occurs in less than 1 percent of all people infected with the human T-lymphocyte leukemia virus-1 (HTLV-1). Most of them never develop any illness caused by this virus. Researchers have been interested in determining why such a small proportion of people infected with HTLV-1 ever become sick, while everyone infected with HIV, a related virus, eventually develops AIDS and dies. One of the hallmarks of HIV infection is that the levels of cytotoxic T-lymphocytes in the blood stream are extremely high. It appears that a similar situation may hold true in those HTLV-1-infected people who develop tropical spastic paraparesis. These cytotoxic T-lymphocytes are equally distributed in both blood and cerebrospinal fluid.

To date, little is known about the role that cytotoxic lymphocytes play in producing, as opposed to curing, disease. In one clinical trial, however, treatment with high doses of the steroid prednisone, which suppresses the production of cytotoxic lymphocytes, produced some improvement in patients with tropical spastic paraparesis. Accompanying the improvement was a drop in the number of cytotoxic lymphocytes specific for the HTLV-1 virus. Lymphocyte counts did not fall in patients who showed no clinical improvement.

Recommendations for the Future

- Develop better ways of identifying potential autoimmune components of psychiatric disorders than the usual screening for "markers."
- Determine whether recognition of neural antigens influences brain development and function.
- Study how T-cell-mediated inflammation may lead to autoimmunity in the nervous system.
- Identify and characterize novel or rare nervous system disorders associated with the immune or autoimmune response as revealed by family studies and/or unusual clinical presentations.
- Stress the importance of probing molecular mechanisms of an entire process, recognizing that multiple points could provide opportunities for therapeutic intervention.
- Develop better ways of distinguishing primary immune etiology from secondary or from exacerbating effects of the immune/inflammatory cascade.
- Create an animal model of autoimmune disease directed against a neuronal antigen, such as is found in human paraneoplastic syndromes.

Further Reading

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NEUROVIROLOGY AND MENTAL DISORDERS

"The impact of viral infections is determined by behavior."
—Linda S. Crnic

Many viruses can infect neurons and remain dormant in them for years.

Viruses have long been suspected of causing behavioral problems, including severe mental illness. This idea has gained new force with the advent of AIDS and AIDS-related dementia. Further evidence comes from the repeated finding that many viruses (neurotropic viruses) infect neurons and can remain dormant in them for years. In addition, the symptoms of neurotropic viral infection share many similarities with both behavioral and neurological disorders. Manic depression and multiple sclerosis, for example, are both relapsing disorders, and the association between these two diseases is tenfold higher than chance.

Moreover, at least some neurotropic viruses display a pattern of latency and reactivation similar to the cyclic pattern of these two disorders. Still, it has proven difficult to confirm the connection between viral infection and behavioral disorders, in large part because such disorders may occur years or decades after exposure to the virus.

Age and gender can determine the severity of damage produced by viral infection.

Investigators have pondered the many ways in which viruses can cause behavioral disorders. One hypothesis is that a common virus, one that infects a large proportion of the population, could injure the nervous system in some extraordinary way in only a few susceptible individuals. Animal studies have shown, for example, that viruses can produce a range of effects, from none to severe, in different inbred strains of laboratory rodents. Other studies have found that the age of an animal or its gender can determine the severity of damage produced by viral infection.

Another possibility is that the host's immune response, and not the virus per se, damages neurons, which would also make it difficult for researchers to link the initial viral infection to the eventual behavioral change. For example, there is sound epidemiological evidence that some viruses trigger the immunological changes that produce multiple sclerosis. Yet many good investigators have spent significant parts of their careers searching unsuccessfully for the offending virus.

Severe behavioral changes could result from subtle underlying damage to the nervous system.

Researchers must also deal with the fact that while the eventual effects of a viral infection on behavior might be severe, the underlying damage to the central nervous system could be subtle. Viral infection may produce changes in neuronal organization, for example, or slight up- or down-regulation of certain host genes. Viruses might also stimulate autoimmune reactivity if viral proteins share antigenic features with important host molecules (molecular mimicry).

Viruses Produce Cognitive Defects

Behavioral changes are often the first sign of viral infection. Though most adults try to deny this, viruses can have major effects on mood, behavior, and mental ability, a fact appreciated by anyone who has cared for a 2-year-old with a cold. In fact, many investigators now believe that pathogens, including viruses and bacteria, must have played a very important role in evolution. While it is more common to think of predators as playing a dominant role in natural selection, more selective pressures were probably exerted by pathogens than predators. Therefore, animals have developed behaviors that lead them to avoid infecting others with pathogens or becoming infected themselves.

Clinical depression may be a normal response that is inappropriately activated.

When an animal is sick, its temperature rises, it stops eating, curls up to conserve heat, and lowers its activity level to conserve energy. These responses reduce its interactions with other animals, limiting the spread of the pathogen. At least some of these changes are linked to the release of interferon-α that occurs during the immune system's response to viral infection. In primates, interferon-α produces changes in sleep patterns reminiscent of those seen in human depression. To some investigators, this suggests that each person is programmed to experience depression in response to viral infection. If this is indeed the case, then perhaps clinical depression is a normal response inappropriately and excessively activated by some wayward biochemical process. Whether this notion proves true or not, it opens heretofore unconsidered avenues of investigation into the etiology of a major mental illness.

A Model for Hyperactivity

Can the aftermath of a viral infection yield a fairly normal animal with a discrete lesion that generates a specific behavioral disorder? Investigators have used a mutant strain of herpes simplex virus to study this question. Newborn mice were infected with the mutant virus, and those that survived became hyperactive. The principle neural lesion in these mice was an alteration in migration of neurons into the cerebellum, producing abnormal development of this part of the brain.

Though the neurobehavioral effect of infection was significant, the infection itself was mild, and the animals' growth was normal in all other respects. All traces of the virus were gone after 20 days, though the hyperactivity was permanent.

Mild infection in newborn mice left permanent hyperactivity.

The hyperactive mice exhibited the same daily rhythm of normal and uninhibited behavior seen in hyperactive humans. Even with the threat of touching an electrified grid, the animals could not resist walking around. These animals could, however, master all other learning tasks, indicating that the viral infection had not diminished their intellectual abilities.

These findings are important for at least two reasons. First, it was not widely considered before that the cerebellum was involved in controlling activity. Second, cerebellar organization occurs fairly late in human development, extending as long as 7 months after birth, and thus could be particularly vulnerable to a variety of insults, such as neurotropic viruses. On the other hand, it is possible that very subtle pathological alterations in other brain regions may be partially or largely responsible for hyperactivity.

Schizophrenia and Influenza: Epidemiological Evidence

Further evidence that viruses can disrupt normal brain development, and thus cause major behavioral difficulties long after infection, comes from epidemiological studies of schizophrenia. This research, conducted largely with the detailed medical records kept by Scandinavian health departments, strongly suggests that an important part of the predisposition to schizophrenia consists of a disruption in fetal brain development. This probably occurs in the second trimester of gestation; some studies have even more closely pinpointed the sixth month of gestation.

The hypothesized disruption of brain development may involve errors in the migration, positioning, orientation, and connection of young neurons. These errors of development may be triggered by exposure to teratogens, such as maternal viral infection, that occurs during a critical period of gestation. The chief viral culprit identified so far is influenza virus.

The first of numerous studies that support these hypotheses focused on women who had been pregnant during the Helsinki influenza

TABLE 2.—Replication tests of the Helsinki "second-trimester" effect

Author	Nation	Finding
Mednick et al. 1988	Finland	Second trimester
Kendell & Kemp 1989	Scotland	Replication for females Replication (6th month effect)
Bowler & Torrey 1990	U.S.A.	Nonreplication
O'Callaghan et al. 1991	U.K.	Replication (6th month effect)
Kunugi et al. 1992	Japan	Replication (6th month effect)
Waddington 1992	Ireland	Replication
Crow & Done 1992	U.K.	Nonreplication
Machon & Mednick 1993	Finland	Replication (6th month effect)
Fahy et al. 1993	U.K.	Replication
Welham et al. 1993	Queensland	Replication
Kendell et al. 1993	U.K., Denmark Scotland	Replication Replication
Beckmann et al. 1993	Germany	Replication

[After S. Mednick]

epidemic that ran from October 8 to November 14, 1957. This was the first appearance of a new strain of influenza known as A2. For comparison, the researchers also studied women who had been pregnant in the 6 preceding years.

The incidence of schizophrenia was greatest when the fetus was exposed to influenza during the second trimester.

Using psychiatric records kept by the eight mental hospitals in the vicinity of Helsinki, the researchers found a significant increase in the rate of schizophrenia for those individuals who had been exposed to A2 influenza during their second trimester of fetal development. Those individuals who had been exposed to the epidemic in their first or third trimesters of gestation, and the controls born in the 6 preceding years, showed no increase in rate of schizophrenia. When the exposed individuals reached 30 years of age, their rate of schizophrenia was more than double the normal lifetime risk for schizophrenia.

A review of the excellent antenatal clinic records in Helsinki revealed that these "second-trimester schizophrenics" were not only exposed to the virus but that obstetrical nurses had noted that the mothers had actually suffered an influenza infection during the second trimester. The antenatal clinic files also made it possible to determine the stage of fetal development based on date of last menstruation. In previous studies, the stage of gestation was estimated from date of birth; this estimate would be thrown off by premature births, which are more numerous among schizophrenics.

These scientists then replicated their findings in a larger investigation involving all births of schizophrenics and all influenza epidemics during a 40-year period (1911-50) in Denmark. Access to data on births of schizophrenics was made possible by Denmark's Institute for Psychiatric Demography, which houses the world's most complete and accurate national psychiatric register. The scope of the study enabled the researchers to further narrow the time of viral insult to the sixth month of pregnancy. The findings of these two studies have been replicated in different parts of the world by several independent investigators (tables 2 and 3).

Further studies pinpointed the sixth gestational month as most vulnerable for schizophrenia.

TABLE 3.—Replication studies covering several epidemics

Author	Country	Period covered	Finding
Barr et al. 1990	Denmark	40 years	Replication (6th month effect)
Sham et al. 1992	U.K.	22 years	Replication
Morris et al. 1993	Ireland	50 years	Replication (3rd trimester effect)
Takei et al. 1993	U.K.	27 years	Replication
Welham et al. 1993	Australia	3 large epidemics	Replication

[After S. Mednick]

Perhaps not coincidentally, the second trimester is a critical time for development of the hippocampus, part of the brain's limbic system. Scientists have long thought that a defect in this area plays a role in schizophrenia. Now, they are using magnetic resonance imaging to see if the brains of Helsinki second-trimester schizophrenic patients show anatomical defects in the hippocampus. They are also examining 6-month-old infants of mothers who have suffered a second–trimester influenza infection to determine whether the infants are defective in neurointegrative functioning.

Chronic Fatigue Syndrome: Repeated Viral Assaults?

Major, persistent, debilitating fatigue is the hallmark symptom of chronic fatigue syndrome, a condition with no known cause or cure. Diagnosis is difficult, based more on the absence of other overlying problems that might cause fatigue, such as HIV infection or Lyme disease, than on a positive laboratory test for some pathogen. Other symptoms include sore throat, headache, chest pain, depression, and dyslogia.

Theories to explain the origins of chronic fatigue syndrome have come and gone, but recent research suggests that the condition begins with an acute viral infection. According to this theory, the initial infection, which may be an influenza-like disease, produces an immune dysfunction. This is not a full-blown immunodeficiency as seen in HIV infection, but rather a small change in the immune system. Subsequent infections—most likely by viruses other than the initial culprit—or other stressors further change the activity of the immune system. The end result is chronic fatigue syndrome.

The link between chronic fatigue syndrome and viral infection has not been established.

The evidence that a viral infection has occurred is sketchy, and the literature does not give universal support to any one virus being either the initial or secondary insult. At various times, retroviruses, enteroviruses, and several herpes viruses have been linked to chronic fatigue syndrome.

Apparently strong evidence seemed to favor roles for retroviruses and enteroviruses in chronic fatigue syndrome, but recent studies of specific viruses have shown that probably neither of these virus families is involved. Double-blind experiments with an HTLV-II-like agent and a human spumavirus found that chronic fatigue syndrome patients did not differ from control subjects on the presence of virus or antiviral antibodies. Similarly, the study of appropriate controls in enteroviral assays indicates that the presence of viral RNA in muscle is not a specific property of chronic fatigue syndrome patients. Thus, the most promising viral candidates for association with chronic fatigue syndrome are currently the lymphotropic herpes viruses.

All the viral candidates produce persistent infection that can reactivate.

Epstein-Barr virus, a member of the herpes family, was the leading candidate in the mid-1980s considered to cause chronic fatigue syndrome. However, investigators came to the conclusion that if Epstein-Barr virus was involved in the syndrome, its involvement was not invariable. Since then, investigators have found some evidence, also not consistent, of a second herpes virus (HHV-6) in the tissues of chronic fatigue syndrome patients. These viruses share several characteristics that would be consistent with their being involved in this disorder. All, for example, produce primary infections leading to persistent infection, which can reactivate to produce another acute infection after immune system activation.

With the evidence of viral involvement equivocal at best, researchers have been asking if viruses play any role at all in chronic fatigue syndrome. While the answer is unclear, there is certainly no proof linking any one virus to the syndrome. However, the immunological evidence remains that people with chronic fatigue syndrome have more antibodies to viruses as a whole than normal individuals. One patient might have a high titer of antibodies to Epstein-Barr virus, while another may have elevated levels of antibody against HHV-6. The numbers of certain subtypes of antibodies and natural killer cells are frequently lower than normal in chronic fatigue syndrome patients. However, since no single measure of immune system status is consistently changed in all patients, researchers are being forced to look further, at cytokine levels, for example, for the underlying cause of this disorder.

A Compromise Response

When cells in the periphery become infected with a virus, the most common response is for the immune system to attack those cells, kill them, and clear them from the body. This is less of an option in the central nervous system, because killed neurons would not be replaced. Evidence suggests instead that the virus is merely inactivated in infected neurons, but at the cost of always having a virus' genetic material present in those cells.

Infected neurons are not killed; the virus is merely inactivated.

Studies of the Sindbis virus provide support for such a compromise. Sindbis belongs to a family of viruses that are spread by mosquitoes and cause encephalitis. These viruses infect mostly neurons, providing investigators with the opportunity to study the immune response that follows a neural infection within the central nervous system. Upon inoculation into mice, Sindbis virus replicates throughout the brain, and an immune system response occurs. Adult mice recover within a week with little evidence of neuronal damage, while newborn mice suffer major nerve cell destruction and die within 3 to 4 days after infection. Because the virus is the same in both cases, the key to this problem must lie in the neurons themselves.

Investigators suspected that a particular gene might be involved in protecting mature neurons. This gene codes for a protein, found in the inner membrane of mitochondria (part of the cell), that by some unknown mechanism inhibits the normal neuronal death that occurs during the later stages of brain development. When inserted into cultured cells that would otherwise die from Sindbis infection, the gene protects the cells from death, though the virus itself is not killed. In fact, the virus continues to replicate in the protected cells, albeit at a low level, and the infection remains persistent for months, during which time the cultured cells continue growing and dividing. Researchers are now looking for

Some viruses continue to replicate at a low level in neurons.

evidence of differential gene expression in mouse neurons as well as for the function of this protein.

The other mystery with Sindbis virus is that genetically inbred mice that lack T- and B-lymphocytes do not show any symptoms of Sindbis encephalitis, though they develop a persistent infection in the nervous system. T-lymphocytes infused into these mice do not clear virus from infected neurons. This may happen because neurons do not express antigen-presenting MHC class-I or MHC class-II molecules on their cell surfaces to signal the need for T-lymphocytes.

Antibodies can suppress viral replication in infected neurons but cannot stop it.

What does seem to have an effect on the virus is adding antibodies removed from normal mice that have previously recovered from Sindbis infection. These antibodies seem to suppress viral replication in infected cells, though the mechanism involved is unclear. What is known is that the antibodies get inside infected neurons. In addition, viral gene expression continues at low levels in infected neurons, even those treated with antibodies that can prevent active viral production or those removed from normal adult mice that had recovered from the infection. Using a new technique that allows amplification of genes in cells, researchers have recently found single copies of other viral genomes in latently infected neurons.

From these results, investigators have postulated that neurons can protect themselves from death only at the expense of maintaining a store of suppressed viral genetic material. B-lymphocytes, producing antibodies against key viral antigens, keep viral replication suppressed for the life of the animal, which might explain why many antiviral antibodies remain in the central nervous system for life—they may be necessary to prevent latent virus from reactivating. If this eternal vigilance fails, the virus may once again appear, which could be a mechanism by which some conditions, such as postpolio syndrome, reappear long after recovery from the original viral infection.

Establishing Infection

Some neurons will take in viruses and incorporate viral genomes into their own.

The development of viral latency has three stages: establishment, maintenance, and reactivation. Investigators have found evidence that the first step is a function of the neuron itself. This was shown using a strain of herpes simplex virus that lacks a key regulatory gene. This mutation renders the virus harmless; though it infects neurons, killing a few, it does not kill the animal and will not produce new viruses. To the surprise of investigators studying this mutant, it does establish a latent infection within neurons but without expressing any of its own genome. For some reason, certain neurons—not all—will take in viruses and incorporate their genomes into their own. This mysterious process is now under active study.

Another virus that establishes latent infection in neurons is the varicella zoster virus. It causes chicken pox during initial infection and can reactivate to produce shingles, zoster, or postherpetic neuralgia, a condition of long-term debilitating pain. Unlike herpes simplex virus, which is harbored in sensory neurons, varicella zoster virus hides in the satellite cells that surround sensory neurons. Another difference is that latent herpes simplex virus expresses only one gene when it is incorporated in the host genome, while varicella zoster expresses several genes. Investigators are now studying these genes to determine their role in the latency and reactivation processes.

Some viruses persistently infect neurons and remain active at all times, rather than entering a latent stage. One example is Theiler's virus, a common virus in mice. This virus enters brain tissue and remains active there for the lifetime of the host, producing a demyelination syndrome. The virus also infects other organs, but the immune system clears the virus from those locations within a few weeks.

Some viruses remain fully active in neurons for the lifetime of the host.

Neural Damage Through the Production of Free Radicals

Many neurotropic viruses indirectly kill neurons by inducing cell-mediated immune responses in the central nervous system. For example, Borna disease virus (BDV), which primarily affects horses and sheep but also might infect humans, stimulates an immune response that inadvertently causes neural damage. At the peak of infection, tremendous tissue damage occurs, which investigators have correlated with levels of expression of proinflammatory cytokines, including interleukin-1, interleukin-6, and tumor necrosis factor-α. The expression of these cytokines coincides with the peak levels of enzymatic activity for inducible nitric oxide synthase (iNOS) in the brain, supporting the notion that certain cytokines may produce central nervous system damage by stimulating macrophages or resident microglia to produce free radicals such as nitric oxide.

During the disease process initiated by BDV, the activity of iNOS dramatically rises in brain regions where viral RNA expression occurs. The correlation of iNOS expression with the development of neurological signs in Borna disease, as well as enhanced expression within brain regions with inflammatory lesions, may indicate that

Nitric oxide may contribute to virus-induced neural damage.

the production of nitric oxide as a toxic substance contributes to virus-induced neural damage.

Nitric oxide can be protective as well as destructive.

The action of nitric oxide, however, is paradoxical; it can be neurodestructive as well as neuroprotective. Nitric oxide-mediated neurotoxicity is engendered by reaction with the superoxide anion that leads to the formation of peroxynitrite, a highly reactive oxidizing agent capable of causing tissue damage. The recent finding that expression of xanthine oxidase, which provides a source of superoxide anions, is also highly upregulated in the brain during Borna disease, supports the concept that free radicals such as nitric oxide may mediate virus-induced neurodegeneration.

Preliminary studies of compounds that either inhibit nitric oxide synthase or scavenge nitric oxide show that a reduction of nitric oxide concentration decreases the severity of BDV infection as measured by the loss of body weight. If further studies confirm these findings, investigators would have a new lead to therapeutic strategies that might ameliorate the effects of neurotropic viruses.

Lentiviruses: Infectious Chameleons

Lentiviruses may have a unique set of genes for growth and replications.

HIV is a member of the lentivirus family, whose members all cause diseases that involve infection of macrophages and microglial cells within the central nervous system. Lentiviruses are capable of replicating in nondividing, terminally differentiated cells, unlike other RNA viruses that can only grow in dividing cells. This suggests that lentiviruses may have a unique set of the genes required for growth and replication—genes normally active in growing cells but quiescent in terminally differentiated cells—that would be good targets for intervention. Such intervention could involve blocking the expression of those genes or interfering with the function of the gene products.

Lentiviruses are also species specific, suggesting that they require specific host cell proteins in the early stages of viral replication. In addition, lentiviruses express significant genetic diversity once infection has occurred. However, investigators have found that those viruses that go to the brain may have a much narrower genetic diversity than do those that infect tissues outside the central nervous system.

Lentiviruses may have acquired some genes from their hosts.

In addition to regulatory genes, all lentiviruses have auxiliary genes that are not required for growth in tissue culture but are necessary for infection *in vivo* and pathogenesis. Some investigators now believe that these viruses, over the course of their evolution, have acquired genes from their hosts that are critically involved in disease progression. These genes may have little in common structurally, but they are similar functionally across all members of the lentivirus family. As such, understanding their role in the viral lifecycle may prove important in understanding the pathogenicity of these viruses.

Using the simian immunodeficiency virus as a model, investigators have begun probing the role of genetic heterogeneity in an AIDS-like disease. The researchers started with a variant of this virus that only infected T-lymphocytes and did not enter the central nervous system. As this virus progressed from one animal to another, its cell-type specificity broadened, first to include macrophages, then eventually to include brain and lung tissue. Accompanying this evolution were changes in two regions of the viral genome. One of these regions coded for viral envelope proteins, which determine the virus's ability to enter a given cell. The other was a regulatory region that controls viral replication once it has infected a cell. These changes were relatively minor, but they were enough to enable the virus to cross the blood-brain barrier, perhaps via infection of endothelial cells, and enter and replicate in neurons.

Minor genetic changes can enable a virus to enter and replicate in neurons.

Human Immunodeficiency Virus

Two key targets of HIV are the immune system and the brain. Not surprisingly, then, individuals with AIDS often show neurobehavioral symptoms in addition to profound immunological deficiencies. However, HIV's effects on the brain and the immune system are not independent of one another. To begin with, HIV appears to enter the brain in infected monocytes/macrophages (fig. 8). In addition, the brain and immune system are linked by a variety of neural and hormonal signals, some of which may be altered by HIV infection.

Autopsy studies have found that the majority of AIDS patients have HIV infecting the central nervous system. However, only a fraction of AIDS patients develop what is known as AIDS dementia complex, characterized by progressive impairment of motor and cognitive functions. The possibility exists, however, that HIV may exert physiologic effects in the brain, via the many connections between the brain and immune system, that influence the course of the systemic infection without impairing brain function.

Most AIDS patients have HIV in the central nervous system, but only a few develop AIDS dementia complex.

During the early stages of HIV infection, few if any symptoms of neurological damage are evident. But even then, neuropsychologists can often measure subtle deterioration in thought processes, including reasoning, memory, and reaction time, and patients themselves

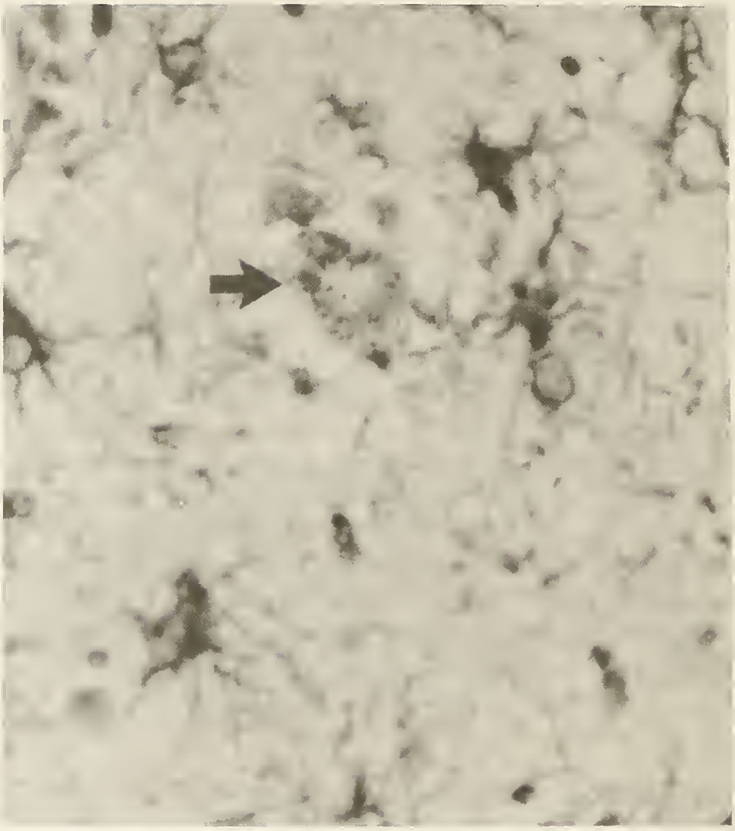

Figure 8.—Photomicrograph of a tissue section of brain from a person who died with AIDS, showing HIV (black grains, center) within a multinucleated giant cell (arrow) that has likely arisen from a monocyte/macrophage. HIV nucleic acids were detected by *in situ* hybridization yielding grains of photographic emulsion visible with a light microscope. The cells surrounding the HIV-infected cell are astrocytes reacting to the infection. Approximate magnification: 1000 times. [Courtesy S. Koenig.]

report slight problems in performing everyday tasks. In this stage, neuropsychological testing may be more sensitive than neuroimaging (such as magnetic resonance imaging scans) in identifying the early and often mild effects of damage in the central nervous system. Recent studies of HIV infection have found a loss of large neurons in the cortex, the brain region involved in many cognitive functions. Other studies have reported that the density of neuron-to-neuron connections is reduced in HIV-infected brain tissue.

Drugs that block calcium flow might prevent HIV-induced neuron death.

Recently, investigators have found that a portion of the HIV's coat protein is toxic to some animal neurons *in vitro*. This protein, called gp120, appears to affect the flow of calcium ions out of neurons, which can cause cell death. This suggests that drugs that block calcium flow might prevent this toxic effect.

Researchers are also finding that other viral proteins can alter immune cell function. One protein, known as gp41, stimulates antibody production by B-lymphocytes. This might explain why many AIDS patients develop hypergammaglobulinemia—a nonspecific activation of B-lymphocytes that then produce large amounts of immunoglobulin with no protective influence. Another viral protein, called gag, inhibits *in vitro* lymphocyte and natural killer cell activity, an effect countered by interleukin-2.

Summary

The past 15 years have seen a renewed focus on the role that viruses can play in producing neurobehavioral disorders, and as a result, it is now clear that viruses infect the central nervous system, damage neurons, and trigger a host of physiological changes in both the nervous system and the periphery. Many of these changes appear to involve the links between the brain and the immune system, and thus viruses are proving to be useful tools in further probing these connections. Learning more about the mechanisms by which viruses interact with the central nervous system will certainly provide new insights into many areas of brain function, including gene expression in neurons, the cross-talk between cytokines and neuropeptides, and development and organization of neural networks within the brain.

Viruses are proving to be useful tools in probing the links between the brain and the immune system.

Recommendations for the Future

- Determine whether viruses are involved in the etiology of mental disorders and chronic diseases of the nervous system; investigate human populations that have been prenatally exposed to viral infections.

- Elucidate brain-specific mechanisms regulating viral replication: tropism, need for viral diversity for infection, latency, activation, and cellular factors continuing viral infection in the brain.

- Determine whether lack of particular immune reactions in the brain may permit propagation of viruses and other pathogens in this organ.

- Analyze how the interaction of the nervous system and the endocrine axis in the immune system determine susceptibility to particular outcomes of virus infection.

- Study viral pathogenesis in animal models to evaluate the differential effects of viruses on the adult and developing nervous system.

- Develop an understanding of how astrocytosis and microgliosis relate to pathological states caused by viruses in adult and developmental models.
- Study the effects of common, mild viral infection on behavior and behavioral adaptations that help to avoid or fight infections.

Further Reading

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TECHNICAL OPPORTUNITIES AND PROBLEMS

"Science plus public-health needs equal opportunities."

—Frederick K. Goodwin

Many consider this period in scientific history to be the Age of Biology, when biological knowledge has reached that critical mass that suddenly propels all fields forward at breakneck speeds. Much of this newfound wisdom and intellectual energy has been made possible by the development of new tools—ranging from the polymerase chain reaction to high-field magnetic resonance imaging—that investigators now have at their disposal. The technical advances embodied in these tools are themselves a product of the Age of Biology, and certainly, further knowledge will only lead to newer, more powerful tools, which will, in turn, propel understanding of biological systems even faster.

This Age of Biology has produced new knowledge and new tools for further discoveries.

Not that these advances will be a panacea. Often, new information, gathered with new tools, raises more questions than it answers. For example, new analytical techniques now enable investigators to detect and measure minute amounts of biologically active substances, such as cytokines or neuropeptides. But finding these substances in increasing numbers of places in the body tells little about what role these substances may be playing, if any, in a particular tissue. Likewise, immunologists have developed ever more sophisticated measures of immune cell activity *in vitro*. However, the relevance of *in vitro* activity to an *in vivo* immune response is not necessarily clear. Thus, investigators not only have to learn what to do with new tools, but also must determine the proper interpretation of the data they may be able to gather with such technological advances.

The proper interpretation of data is a major challenge.

In this context, the National Institute of Mental Health's advisory panels on psychoneuroimmunology and neurovirology were asked to identify key technical opportunities, methodological problems, and potential breakthroughs in knowledge that are emerging now and will mature over the next 5 to 10 years.

A Need For Functional Anatomy

Very little is known about the circuitry that connects the nervous and immune systems.

A key piece of information needed to better understand the links between the nervous and immune systems is the circuitry that connects them. Amazingly enough, very little is known about these anatomic and biochemical connections. For example, substance P is known largely as a sensory neurotransmitter, that is, it is a neurotransmitter in sensory neurons. However, there are substance P-containing neurons in the autonomic nervous system that innervate the spleen, though not much is known about the function of these neurons.

It is far from easy to trace neural and biochemical circuits through the body. Many biochemicals, for example, are able to diffuse a considerable distance through the intracellular matrix from their point of release. Therefore, localizing a store of neurohormone, for example, does not always pinpoint those neurons or immune system cells where that substance acts. Therefore, mapping studies must include efforts to determine the distribution of both biologically active substances and their receptors.

A Possible Vector Into the Brain

Investigators studying the interaction between neurons and the herpes simplex virus have discovered a mutant virus that establishes a latent infection in neurons but does not replicate in or kill these cells. The mutant virus is called HSV4-minus. In addition, the virus only infects neurons. Even though this virus is not pathogenic, it does incorporate itself permanently into neuronal DNA. Thus, this variant of herpes simplex is the first nonpathogenic virus known to target nerve cells and could serve as a vector for delivering genes into the nervous system.

A mutant virus may be used to carry large amounts of DNA into neurons.

One useful trait of this virus is that it can carry relatively large amounts of extra DNA into a cell. It may be possible, then, to incorporate not only structural genes that code for one or more proteins, but also the natural regulatory genes that control the expression of the structural genes. The structural genes that scientists would want to increase the “dosage” of are, for example, those encoding proteins that increase the survival of aging neurons.

Model tests, using a reporter gene coupled to a regulatory segment from another gene, showed that the recombinant virus latently infects spinal neurons. Furthermore, the reporter gene does turn on, and the infected cells do make reporter gene product for extended periods of time. Currently, investigators are working to couple the gene for nerve growth factor with a regulator gene and inserting the combination into HSV4-minus. Augmenting nerve growth factor in neurons could perhaps help protect neurons against the damage associated with Alzheimer's disease and other neurodegenerative disorders.

The stumbling block at present is finding a promoter element that will remain active when the viral infection passes from the acute to the latent stage. Investigators working with these viruses believe that there are no theoretical constraints; they just have not found the correct combination of promoter and placement within the viral genome.

There are no theoretical constraints in using a virus as a vector into the brain.

Mouse Model for Studying HIV Gene Expression

As its name implies, the human immunodeficiency virus only grows in humans, presenting a challenge to investigators looking for weaknesses in the virus' defenses against elimination. Using the techniques of genetic engineering, investigators have inserted several of HIV's regulatory genes into mouse nerve cells and are now attempting to determine what molecular events trigger expression of these genes and how these genes' products affect cells that make them.

The investigators were interested in how changes in promoter elements might affect replication of HIV in the central nervous system, so they inserted into the mouse genome a reporter gene coupled to promoters from three different HIV strains—one that only infected T-lymphocytes and two that were isolated from brain and cerebrospinal fluid of a patient who died with AIDS dementia. They can now use these transgenic mice to study the molecular events that lead to HIV activation.

Several HIV regulatory genes have been inserted into mouse nerve cells.

Early experiments showed that the promoter region taken from the HIV strain that only infects lymphocytes did not turn on in brain cells. This suggests that a virus' apparent tissue specificity, or tropism, involves more than its ability or inability to infect a particular type of cell. Indeed, a virus with a narrow tropism may infect many cell types, but its genome may then fail to express itself once the infection process is complete. In this case, some molecular factor in neurons, as opposed to lymphocytes, may be preventing the promoter taken from a lymphocyte-tropic HIV strain from working outside of its normal environment.

In contrast, promoters from the two neurotropic strains did express in brain cells, and were most active in areas rich in neurons, such as the hippocampus and the nuclei of the visual system. Regional patterns of expression between the two neurotropic strains differed.

Investigators pursing these leads are now hoping to identify the factors that influence gene expression in different strains of HIV and to determine which regions in regulatory genes are activated. In this way, they hope to uncover the cellular factors in the central nervous system that are important for activating latent HIV. This information may, in turn, suggest ways of shutting down the transcriptional process, and thus, the virus' pathogenicity.

A New Kind of Genetic Analysis

Thousands of nucleic acid fragments covering millions of base pairs can now be separated.

A standard method of separating proteins from a mixture is to apply a sample to a gel and electrophoretically separate the proteins in two dimensions. Now, the same principle has been applied to nucleic acid fragments. In two-dimensional "electrophoretic genetics," nucleic acid fragments are separated in the first dimension according to fragment size and in the second dimension according to sequence through the use of a chemical denaturing agent. This technique can simultaneously separate thousands of nucleic acid fragments covering millions of base pairs. The difference between fragments can be as small as a single nucleic acid.

As a result, investigators will be able to more easily study genetic polymorphism—the natural variation across individuals of a species or even across cells within the same individual—that occurs in the base-pair sequence of a particular gene. When combined with amplification techniques, such as polymerase chain reaction, two–dimensional electrophoretic genetics will enable investigators to study gene expression in individual cells.

Multigene defects found in mental diseases and mutations involved in nerve degeneration and aging can be examined.

Using this new technique, investigators should be able to map multigene defects that are implicated in many mental diseases. The exquisite single-nucleotide resolution should also make it possible to look for mutations involved in producing changes seen in nerve degeneration and aging. In addition, it should now be much easier to study the genomic rearrangement that occurs as part of the maturation of both B-lymphocytes and T-lymphocytes and may, if recent experiments are confirmed, also occur in the nervous system during neuronal differentiation. This technique may also prove to be a complete way of looking at total cellular gene expression.

The ability to examine genetic expression in single cells should be a boon to investigators, for it should enable them to discover whether viral and cellular genes are being expressed in single cells in a particular disease state. Researchers will also be able to examine individual neurons and glia to look for evidence of viral latency in regions of the brain that are relevant to a particular disease.

In Situ Polymerase Chain Reaction

In the 1980s, molecular biologists developed a powerful method for making millions of copies of nucleic acid fragments from as little as one copy, thus providing a way of "amplifying" DNA and RNA. This technique, known as polymerase chain reaction (PCR), enables investigators to study the genetic makeup of single cells and is proving to be a powerful new tool for finding viral DNA incorporated into neurons and immune cells. In fact, researchers have already used PCR to detect HIV *in situ* in individual lymphocytes and macrophages.

PCR enables investigators to study the genetic makeup of single cells.

In the past, latent viral genomes, such as those of HIV, herpes simplex, and other neurotropic viruses, have been invisible, both to the cell and to biologists searching for evidence of infection. That is no longer true, because PCR enables investigators to make millions of copies of a single cell's genetic material and then probe this relatively vast quantity of material for viral DNA using standard gene probes; these probes are not capable of identifying a single copy of a nucleic acid fragment.

Combining PCR with immunocytochemistry, a technique called *in situ* PCR, will enable investigators to visualize these cells in tissues as opposed to isolating the cells and testing them *in vitro*. This can have several applications. Detecting mRNA produced from viral genes will give investigators insight into the processes of latency and reactivation by allowing them to identify those gene products necessary for the various stages of the viral lifecycle. In addition, *in situ* PCR will identify types of cells that are infected with a virus, providing information about the ways different cell types restrict/permit viral expression. PCR-amplified mRNA, in combination with two-dimensional electrophoretic genetics, can also aid in the phenotypic characterization of individual neurons and immune cells by providing a fingerprint of a cell's genetic expression.

Cells can be examined in tissues rather than isolating them in test tubes.

Problems With Reproducibility

Often, when a new technique spreads throughout the scientific community, each investigator personalizes the method to reflect the instruments and reagents at hand. Though these changes may seem minor, they can introduce confounding factors that can make comparisons among laboratories difficult if not meaningless. This seems particularly true with both neurochemical and immunological measures, many of which attempt to quantify small differences in naturally variable systems. To address this problem, a number of scientists have suggested that standards be developed for various neurochemical and immunological assays against which all researchers could compare their results.

Such an effort has been started with cytokines. The National Institutes of Health has had a longstanding program for exchanging reference reagents. A researcher can, for example, obtain a reference standard of interleukin-2 with which activity of laboratory samples can be compared.

Another problem in assessing immunological variables is that differences in the overall state of an animal's health may have a greater effect on immune cell activity, cytokine levels, and other measures than any experimental manipulations. Animal care facilities are well maintained, but it is impossible to have each facility exactly as clean as all others. Such variations could confound some interlaboratory comparisons, even those done on the same strain of inbred mice with the same reagents.

Recommendations for the Future

- Create animal models that recapitulate some signs and symptoms of psychiatric disorders.
- Define molecules that reflect brain-immune interactions to study the linkages between these systems and behavior.
- Study immunodeficient mutant mice with characterized genetic defects to determine whether common genes control functioning of the central nervous and immune systems or whether defects in the immune system then affect the central nervous system.
- Develop methods for physiologically (as opposed to genetically) knocking out certain specific cell types, hormones, or cytokines.
- Develop stable lines of phenotypically homogeneous, terminally differentiated neurons, suppressor T cells, microglial cells, and astrocytes that approximate the normal cells.
- Establish standard assays *in vivo* and *in vitro* to evaluate mechanisms of neuronal damage and destruction with a goal of identifying specific molecular pathways that mediate neuronal

damage and correlating the damage with electrical, physiological, and behavioral dysfunction.

- Develop stable, covalent receptor ligands that would permit not only identification, but perhaps modulation of these receptors.
- Establish banks of rapidly obtained tissues and sera from humans with viral infections (e.g., HIV-1, CMV, HSV, HZV, enteroviruses, measles, shingles) accompanied by medical histories, cognitive evaluations, and routine pathological stains of tissue sections.
- Analyze the presence and functional significance of sexual dimorphism at critical points of neuroendocrinimmune circuits.

NEW PERSPECTIVES

Psychoneuroimmunology has already generated novel therapies that are being tested in humans.

Over the past decade or so, investigators in a host of disciplines have been generating new, exciting findings about the many links between the nervous system and the immune system. As this knowledge base grows, it becomes increasingly clear that understanding the physical connections between these two systems, as well as the barriers that keep them separate, has tremendous implications for improving human health. Already, the field of psychoneuroimmunology has generated novel therapies, both behavioral and biochemical, that are being tested in humans. One example is the use of social support therapy to help patients combat breast cancer. Another example is a current trial using nonsteroidal anti-inflammatory agents in treating Alzheimer's disease. Many more are in the pipeline for future clinical trials.

Despite the tremendous strides forward, more is unknown than known about the two-way interactions between the body's two great integrative systems, the nervous system and the immune system. At the molecular level, investigators are almost overwhelmed by the dozens of chemicals that pass messages among the different components of these two systems. Researchers speak of a "unified field theory of chemical communication," one that would reveal the common mechanism used by hormones, neurotransmitters, neuropeptides, cytokines, and their receptors. It is hoped that such a unified theory would make sense of the seeming redundancy and overlap among the various chemical messengers and uncover new avenues for therapy.

In some ways, neural cells resemble immune cells in both function and form.

At the cellular level, scientists are struggling to sort out the roles that the various cell types play in mediating interactions between the two systems. In at least some ways, neural cells resemble immune cells in both function and form, suggesting a common evolutionary beginning. Both classes of cells have the ability to form memories of experiences of the world outside the organism, a property that is seemingly unique among these cells of the body.

At the behavioral level, the meeting ground between the outside and the inside worlds, researchers are more certain than ever that common mechanisms exist to translate experience into action, whether it be the firing of a neuron or the activation of an immune cell. Then again, they are perhaps more uncertain about what those mechanisms may be. What is undeniable, though, is the tremendous variety and richness in the way the body translates stress, be it immunological or psychological, into biochemical actions intended to protect the body from harm. On occasion, these biochemical actions can have the exact opposite effect, causing illness. Again, elucidating these mechanisms, and their weaknesses, offers the promise of great therapeutic advances.

The nervous and immune systems are incredibly complex, and their interaction and coordination adds many more layers of complexity.

This promise, as well as the formidable intellectual challenge, pushes investigators to answer these and other questions. The challenge is so great because, by themselves, the nervous and immune systems are incredibly complex. Each is a hierarchical system in which the various components are able to receive data from several sources, analyze them in an integrative way, and produce an action that represents more than just the sum of individual molecular and cellular events. Then, these two systems coordinate their actions in yet another integrative interpretation of incoming data, producing a situation that is many orders of magnitude more complex than if one were simply studying the actions of one cell or one neural circuit.

A good example of this complexity is the remarkably redundant network by which stressful events suppress a variety of immune responses. This network has multiple pathways by which the same effects on these immune responses are mediated. The network effectively preserves this suppressive response in the face of a variety of situations that might block one particular pathway or another. Evidently, this response is somehow important to the organism, as indicated by the power and redundancy built into the network. Studying one pathway in this network may provide a coherent picture of the relationship between stress and immune responsiveness, but it would in no way be the complete picture.

Development and Function

Because the nervous and immune systems have so much in common, investigators hope to gain some insights into the mechanisms they share by comparing how these two systems developed throughout evolution. One hypothesis holds that these two systems began with the evolution and diversification of the family of genes that control immunoglobulin, or antibody, formation. There are hundreds of members of this family. Parts of these genes are conserved throughout the family, while other segments are variable, enabling the system to interact quickly with a wide range of foreign proteins and other molecules that are not identified as self. The gene products themselves are unique because they resist the actions of enzymes that degrade most proteins, making them suitable for their role of sitting on the outside of cells and mediating intracellular communication. As evolution proceeded, this family of genes diversified, producing many key molecules, such as receptors, in both the immune and nervous systems.

Another evolutionary avenue may have led to the development of the genes for the major histocompatibility complex and other recognition molecules thought to play key roles in both the immune and nervous system. In the immune system, recognition molecules and cell adhesion molecules are central to the separation of self from non-self and for directing the immune response. In the nervous system, such molecules are thought to control the development of the brain itself by controlling the migration of neurons and glia and the formation of connections between neurons. Understanding the development of such a complex cell-recognition system has great importance in understanding the functions of both the immune and the nervous systems and should provide new insights into shared features and common mechanisms.

Understanding the complex cell-recognition system has great importance for understanding both the immune and the nervous systems.

Multiple Pathways

Another common feature of the nervous and immune systems is that of multiplicity. At the system level, there are usually multiple pathways by which a given action, be it an immune or behavioral response, can occur. Indeed, many pathways seem to connect these two systems, as shown by how the effects of stress are transmitted from brain to immune system and back. At the molecular level, both neurons and immune cells are able to integrate multiple chemical signals into an eventual action. In fact, it takes many chemical signals to fully activate a lymphocyte, just as it takes many incoming neurotransmitter and neuropeptide signals, some stimulatory and others inhibitory, to fully activate a neuron.

Many pathways connect the nervous and immune systems, and much is unknown about the different levels of multiplicity.

Much is unknown about these different levels of multiplicity. Investigators have found some of the common mechanisms and duplicate systems. However, no one believes that they have found all the mediators—be they cytokines, neurotransmitters and neuropeptides, or hormones—and the receptors for these substances used by the two systems. Nor have they uncovered all the neural and hormonal pathways that connect the brain and immune system. A key effort, then, must be to identify all the actors involved in this complex dance within each system and between the two.

Researchers also face the task of sorting out genesis and function of these molecules and receptors. When immunologists first discovered cytokines, their initial idea was that one cell type would make one class of cytokine—lymphocytes would make lymphokines, monocytes would secrete monokines, and so on—which would then act on another particular cell type, regulating one particular gene. Today, they know that this is wrong and that there is tremendous overlap in the production of cytokines, the targets they activate, and the actions that result from those interactions. Despite this duplication, it appears that common pathways, mediated by so-called second messengers operating inside cells, translate cytokine binding to a target cell to that cell's activation. Therefore, understanding these second-messenger systems could help unscramble some of the confusion resulting from the overwhelming number of molecules and pathways that investigators are now characterizing.

Heterogeneity in the nervous system is poorly understood.

The same can be said of the nervous system. For example, investigators have found that in one small section of the frontal cortex, a subset of astrocytes expresses the gene for interleukin-1, others express transforming growth factor beta one, a third subset expresses both genes, and a fourth type expresses neither one of these cytokines. Such findings raise the questions: What makes this heterogeneity? What controls it? Is this heterogeneity biologically significant, or is it just an artifact of researchers' inability to subtly probe and detect small amounts of cytokines in some cells in the functioning central nervous system?

In Vitro Versus In Vivo

As in most areas of biological research, investigators carry out the majority of their experiments by isolating a process under study *in vitro*. Whereas working *in vitro* is certainly necessary, the problem is to interpret these results in relation to other processes occurring simultaneously *in vivo*. In other words, does a change in T-lymphocyte activation detected by a particular *in vitro* assay have anything to do with a change in the body's ability to fight a pathogen that the body was exposed to before the T-lymphocytes were isolated?

The answer to this question is currently unclear, but this concern for biological relevance is constantly nagging those researchers who study complex systems such as the immune and nervous systems. These investigators are well aware that *in vitro* studies are essential for elucidating possible physiological mechanisms, but they also know that it is critical to continually check these results against biologically relevant human health outcomes, such as the ability to ward off an infection. It is now clear, for example, that the immune system is relatively stable; that is, it takes a number of factors to trigger its activity. Thus, the relevance of any one change measured by an *in vitro* assay is unclear, and placing too much importance on one such measure may take research down the wrong path.

In fact, this is a major problem in psychoneuroimmunological research. So many variables are changing at any one time in either the brain or immune system, let alone in both at the same time, that it is extraordinarily difficult to understand how the individual pieces fit together. There is a critical need to find ways of both integrating individual findings and studying multiple physiological events at the same time.

So many variables are changing at any one time that it is difficult to fit the individual pieces together.

Fortunately, there are ways of approaching these complex problems. Breakthroughs in molecular biology and genetics offer researchers the opportunity to alter entire systems at once with various genetic lesions. For example, a genetically altered strain of mouse that lacks those lymphocytes that respond to antigens provides investigators with the ability to study other parts of the immune system with one fewer confounding factor. Antisense genetic probes, which can bind to and inactivate targeted mRNA, thereby knocking out a specific protein from key biochemical processes, provides investigators with a new probe of that protein's function. Similarly, chemical agents capable of destroying specific peripheral neural circuits allow researchers to probe subtle, but widespread, changes in immune function that may occur as a result of such neuronal cell loss. These studies may also identify redundant systems that were previously unknown.

But such studies must again be brought back to issues concerning biologically meaningful outcomes, for example, those relating to human health. One way of doing that would be to examine patients with rare neurological, immunological, or psychological disorders for some of the same genetic or biochemical defects being studied in animals. These patients may offer researchers unique opportunities to connect animal models to human disease states.

Studies must ultimately lead to biologically meaningful outcomes.

The key to all of these efforts is to remember that no one technique will provide all answers to all questions. Instead, investigators need to take a variety of approaches, getting as much information as they can from intact animals, isolated organs, cultured cell lines, and disassociated receptors. Multidisciplinary research such as this requires a broader knowledge base than many scientists now possess; it is difficult at best to keep up with results and techniques used by one's peers. Thus, it may be necessary to create new modes of training researchers, enabling them to learn about advances made by researchers outside their limited disciplines.

What happens in test tubes and animals may not hold true for humans.

At the same time, investigators must remember that what happens *in vitro* or even in the most cleverly designed animal model may not hold true in humans. This is probably most true when it comes to studying behavior, for it is extremely difficult to develop animal analogs that truly represent some human behaviors. To accomplish this, investigators will have to determine what processes need to be modeled and then try to create animals with those traits. Again, those with the broadest knowledge of techniques developed across the biological sciences will have the greatest chance of developing such models.

Extreme Versus Moderate Stimuli

Perhaps the hallmark of the majority of complex biological systems is their ability to respond to subtle changes in their surroundings. This is particularly true of both the immune and the nervous systems. Yet it is beyond the ability of most experiments, particularly those done *in vivo*, to recreate subtle stimuli whose effects can be measured. For example, many important studies involve knocking out the particular genes or even entire neural circuits—the former with antisense probes, the latter with neurohormone toxins—and measuring the effect on an animal's health. Certainly, those studies are important, and they may even provide insights into some human disorders. However, it is likely that most human maladies result from slightly abnormal changes in the normal response, and these "brute force" experiments may not help get at the root cause of these maladies.

It is beyond the ability of most experiments to recreate subtle stimuli whose effects can be measured.

Another aspect of this problem is the difficulty researchers have in measuring minute quantities of biochemicals *in vivo*, or administering physiologically important concentrations of such compounds into biologically relevant regions of the brain. This is a particular concern when studying such potent compounds as cytokines and neurotransmitters. In all likelihood, these compounds are produced close to where they act. Therefore, infusing cytokines into the cerebrospinal fluid may produce biological effects that have no bearing on real circumstances simply because the effects of flooding the brain with a cytokine might overwhelm small, but real, biological effects. However, as biological techniques improve, investigators will be able to measure smaller, more localized effects, and most believe that further advances will soon enable investigators to conduct such experiments on a physiologically relevant scale.

Defining Stress

Stress has become chief psychological variable in the field of psychoneuroimmunology, and the effects of stress on immune competence have provided some of the most compelling evidence supporting the link between the brain and immune system. But stress is a broad, inexact concept, with no firm definition. As a result, many investigators in the field are concerned that without a standard definition of stress, it is difficult at best to compare results from different experiments and laboratories.

Stress is a broad, inexact concept, with no firm definition.

For example, stress can be chronic or acute. It can be physical or psychological. What is stressful for one animal might not be for another, in the same way that some humans cope better with stress than others. Investigators themselves are now trying to cope with the stress of not having good operational definitions of stress.

Some say that the problem with stress is that the biological response has nothing to do with the stressor itself, but rather with an animal's characteristic way of coping with the stressor. In some situations, the coping response might be activation of the hypothalamic-pituitary-adrenal axis, whereas in a different situation—or even the same situation at a different time in the animal's life—the response might involve suppression of the sympathetic nervous system.

The biological response to stress may have nothing to do with the stressor.

Measuring Appropriate Behaviors

An equally pressing problem involves deciding what behaviors are appropriate to study in the context of psychoneuroimmunology. Behavior can be affected by factors that are only indirectly affected by stress or illness. A sick or stressed animal, for instance, may not eat, and nutritional imbalances may affect behavior. Sophisticated behaviors are often influenced by sensory, motor, or motivational deficits, common sequelae of various infections.

Often, researchers use learning as a behavior that they can alter with some experimental paradigm. But learning may, in fact, be extremely insensitive to manipulation because it is so important to the survival of an animal; one that cannot learn where to find food will soon die. Instead, a more useful measure, though one that will take more resources to acquire, might be to simply observe an animal throughout the day in its acclimated environment and see how various manipulations change those overall behavior patterns.

Gender Differences

Physicians have long noted profound gender differences in the occurrence of many disorders, but particularly autoimmune diseases—an indication that hormonal differences may affect those disease processes. Yet few investigators account for or use sex as an experimental variable, and thus they may be missing an important opportunity, for sex hormones are one of the best understood and most easily manipulated systems in biology. Changing levels of sex hormones early in development, for example, can have profound influences on neuronal organization and thus could serve as an important, well-defined probe for neuroscientists and immunologists.

Better understanding of gender differences may lead to more effective therapeutic approaches to various illnesses.

Some responses are independent of gender, while others are not. Probing these differences may provide important clues to mechanisms involved in communicating within and between the nervous and immune systems. It is also important to relate sexual dimorphism to human illness in order to shed light on how sex hormones may affect response to immunological or neurological stimuli. Ultimately, better understanding in this largely unexplored area may lead to more effective therapeutic approaches to various illnesses.

Therapeutic Possibilities

There is tremendous excitement among those studying the interface between the brain and immune system as they see their research offering new approaches to disease therapy. Recently, for example, investigators found that the hormone prolactin enhances the immune response and have evidence implying that this hormone may aggravate autoimmune diseases. This suggests that a drug blocking prolactin's effects on the immune system may benefit sufferers of multiple sclerosis or rheumatoid arthritis. Similarly, researchers now believe that excess cytokine production may be producing some, if not all, neurobehavioral symptoms associated with AIDS. Since α-melanocyte-stimulating hormone is known to block the effects of cytokines in the central nervous system, it might be possible to use this natural peptide to ameliorate at least one set of symptoms of this devastating disease. With the discovery that the complement system may be involved in producing the cell death associated with Alzheimer's disease, investigators are now searching for drugs that can block this pathogenic mechanism in the central nervous system.

Examples such as these, though just the beginning of the process of moving from discovery to clinical use, have encouraged researchers to begin thinking of likely targets for intervention. High on the list of many investigators are the autoimmune diseases, since many neurochemically and immunologically important mechanisms involved in these diseases have already been identified. Efforts to target key receptors and chemical mediators involved in these illnesses are already underway.

As researchers learn more about the genes and receptors involved in certain disorders, it may be possible to develop ways of controlling the expression of those genes and receptors. Antisense RNAs, which bind to messenger RNA and block the translation of genetic information into protein, may prove to be useful drugs. So, too, might antibodies or other compounds that bind to particular receptors or regulatory proteins.

Antisense RNAs, antibodies, and other compounds that bind to receptors may prove to be useful drugs.

Behavioral Interventions

Since behavior affects immunity, it is likely that behavioral intervention could provide a relatively simple—and inexpensive—method of augmenting the immune response when needed. Already, investigators have shown that social support boosts immunity against various viral infections and also prolongs survival in certain cancer patients. The old expression, "Take two aspirin and call me in the morning" might become "Take two aspirin and call your best friend right now."

What is needed to corroborate these results are long-term longitudinal studies that carefully examine the effect of behavioral therapy by itself or in combination with pharmacological interventions. The recent finding that the placebo effect is larger than previously thought highlights the urgency of this matter. Only with further study, which will require significant commitment of time and money, can the likely benefits of behavioral therapies be realized and used with confidence.

Longitudinal studies that examine the effects of behavioral therapy are urgently needed.

An increasing number of Americans are already convinced that the mind can hold significant control over the rest of the body. These everyday citizens, many highly educated, are turning to alternative forms of medicine that promote the use of behavioral adjustments as a means of maintaining health. It behooves the research community to assess these therapies, for if they work, not only will new avenues of research open up for further studying the interactions between the nervous and immune systems, but all people will benefit.

Recommendations for the Future

There is little doubt that uncovering the connections between the brain and immune systems is one of the foremost challenges of the medical sciences, one that will require the best and brightest applying all their combined knowledge. Already, interdisciplinary teams of scientists have made important discoveries of how HIV, for example, attacks cells in the brain, goes dormant, and becomes activated again by cytokines; how the body's stress response system influences and is influenced by the immune system; and how cognitive therapy can induce changes in biochemical functions that then prolong survival of patients with cancer. Investigators have discovered a few of the many forms of cross-talk between the body's two great integrative systems, and they are learning that there may be a unified form of chemical communication that operates throughout the body. Perhaps as importantly, with each of these discoveries, researchers are breaking down the intellectual barriers that once kept apart the many disciplines that today form psychoneuroimmunology.

The nervous and immune systems together enable the organism to maintain stability in the face of an ever-changing world.

Yet these are just beginnings that have aroused the curiosity of the biomedical research community. After all, the nervous system and the immune system provide the means by which the body interacts with and reacts to the outside world. Somehow, these two systems enable each organism, itself a complex being, to maintain its robust homeostasis, that stability in the face of an ever-changing world that is a hallmark of life. Probing the links between these two systems enables investigators to more closely examine key questions facing all biologists, such as: How do cells communicate with one another? How do they recognize one another, bind to one another, or avoid one another? How do genes, via mRNA and protein, affect not only what goes on inside a cell but how that cell interacts with all other cells in its universe? How do cells, or collections of cells, integrate information and produce the complex reactions that constitute life? We have yet to see the answers to these questions, but if progress continues at the current pace, those answers may come sooner than we ever thought possible.

For progress to continue, the advisory panels made some recommendations requiring cooperation of several institutes and agencies.

- Determine the behavioral effects of immune modulators on humans enrolled in either ongoing clinical trials or in small trials set up for that purpose.

- Introduce appropriate behavioral testing batteries in phase I clinical trials.

- Verify the biological and functional importance of specific gene products.
- Analyze the genetic polymorphism of laboratory strains of rats and mice so that neurobiology being done in rats and immunology being done in mice can be reconciled.
- Overcome undue restrictions on distribution of and access to mutant animals, reagents, cloned genes, and so forth that have been generated with public funding and thus should be in the public domain.
- Develop an inventory and/or repository of available transgenic and knockout mutant mice and rats so that all investigators would have ready access to them.
- Create and distribute standardized materials and methods for easy use of established animal models in a variety of laboratories for verification and elaboration of findings.
- Train graduate students and postdoctoral fellows to integrate the traditional medical disciplines of virology, pathology, contemporary molecular biology, genetics, and neuroscience.

The gauntlet has been thrown down, and scientists have joined the fray, armed with the latest technological advances and the knowledge that is pouring out of the world's biomedical laboratories. This is, after all, the Age of Biology, and the future of psychoneuroimmunology is bound to be full of excitement and great triumph.

Human health will be better for it.

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